An Experimental Study of the Response of the Fat Content of the Blood to Exercise in Health and Diabetes, with some Clinical Observations on the Fasting Level of the Blood Fat.

by

James Waters Thornton Patterson

M.B., Ch.B., M.R.C.P. Ed.

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INTRODUCTION: (1) Aims and Scope.

It must be admitted at the outset that the investigation of this subject was undertaken with no definite and clearly marked course of study arranged. The whole subject of the rôle of fats is so indefinite and obscure and the results of experimentation so widely varying that it was somewhat difficult to know where to begin.

The earliest investigations were pursued with the intention of determining the fasting level of the blood fat in "normals" and in patients suffering from diabetes mellitus, nephritis, obesity, etc. It was felt that the variations in these cases examined early in the investigation might be due to lack of standardisation of diet and consequently the effect of low fat diet and high fat diet on the fasting levels of these patients was next investigated. In two or three cases fat analysis of the stools was also carried out. Nothing of a very definite nature resulted from this line of investigation and the next step was to determine the rise and fall of the fat in the blood after a meal.

Only one case of this type was studied as patients have a definite objection to having blood withdrawn hourly for six or seven hours and become restive if the investigation is pursued, as it should be, over twenty-four hours with a corresponding period/

period of rigid fasting.

It was during the investigation of the effect of low and high fat diet on the fasting level of the blood fat that the author made the observation which opened up what proved to be the main line of this inquiry. During an experiment upon himself in which, while he was on a constant diet, fasting blood samples were withdrawn each morning, it was noted that one sample was unaccountably lower than those samples drawn on the previous and following days. Reflection showed that in the afternoon preceding the day on which the low fasting level was recorded, the author had indulged in a strenuous game of hockey.

The question at once arose did the two phenomena - the strenuous exercise and the low fat level stand in the relation of cause and effect?

Consequently after withdrawing a fasting sample one morning he proceeded to run round the hospital for a quarter of an hour and then withdrew another sample and was surprised to find that the sample withdrawn immediately after exercise showed an increase of almost 100% in the fat content as compared with that in the fasting sample before exercise.

Here, at last, was something definite and this result was confirmed in later investigations.

It was felt that some of the variations in fasting levels found in the earlier part of this investigation might be accounted for by this exercise/

exercise effect and thus in Section I will be found a series of fasting levels in (1) "normals", (2) patients suffering from obesity and (3) patients suffering from nervous diseases associated with tremor. In these cases due attention has been paid to the exercise effect which was eliminated as far as possible by having every patient lying quietly for one hour before the fasting sample was withdrawn.

Section II deals with the effect of exercise on the fasting level of the blood fat in normals and Section III with the same exercise effect in diabetics.

(2) The Importance of the Subject.

The paramount importance of the subject is so obvious that little need be said to justify an investigation along this path.

The vital part played by the fats in diabetes mellitus is a subject well known to all nowadays and the oft repeated maxim that "acidosis kills quickly glycosuria slowly" shows the danger of diabetes to have shifted from carbohydrate to fat metabolism.

The obscurity surrounding the question of adiposity and the innumerable advertisements of systems, medicines and mechanisms for "reducing" show most plainly how important this aspect of the subject is to the lay mind and how incompetent we, the medical profession, are to deal with it.

Similarly/

Similarly with the mechanism of the more definitely pathological types of adiposity our knowledge is at the best obscure.

From the physiological standpoint the mechanism of the digestion, absorption and excretion of fats is comparatively well established. The intermediate stages between absorption and excretion are still far from being thoroughly understood. Though it appears probable that fatty acids are oxidised in the main by a process of β-oxidation we can give but unsatisfactory answers to such questions, as:

(1) Does fat function as a source of carbohydrate? A question of great importance from the point of view of muscle physiology.

(2) What factors control the transport of fat?

(3) In what form is it utilised?

(4) What is the meaning of the variations in the partition of fat between the corpuscles and the plasma?

- and so we may go on.

From the clinical standpoint one can produce a similar series of problems urgently requiring solution and any investigation undertaken to shed light on any part of the fat problem is surely most amply justified.

(3) Difficulties and Complications.

In the first place the technique of fat estimation could hardly be described as simple and elaborate precautions have to be taken to avoid contamination by CO_2 from the air which, if disregarded, throws out the results most hopelessly. The investigator lives in an atmosphere where CO_2 is an evil spirit against which he protects his apparatus and solutions with a holy zeal. All manipulations have to be performed with this CO_2 factor in constant view and constant watch has to be kept against the intruder.

Standardised solutions must, of course, be absolutely accurate and during the various boilings and evaporatings of the alcohol-ether mixtures extreme care must be exercised in order to avoid any spurting or boiling over. This means almost constant supervision.

It took two months of practice to master the technique sufficiently to warrant any experimentation upon the results of which any emphasis could be laid.

The difficulty of obtaining "normals" was finally overcome by the wonderful response of first and second year medical students to an appeal for volunteers. Prior to this, experimentation upon "normals" in the wards - chiefly convalescent patients - met with such variable results that nothing/

nothing of a definite nature was obtained.

To assume that patients on "convalescent diet" in different wards are on a comparatively standardised diet is quite misleading for the term is apparently one of infinite elasticity and not one on which accurate dietetic investigation can be based.

Again it was only latterly, as already explained, that the significance of the effect of exercise on the blood fat level was appreciated and consequently the earlier variations may be due to insufficient supervision in this respect. Convalescent patients in hospital are usually doing odd jobs about the ward and this may have resulted in varying the fasting blood fat level in different cases.

It was considered necessary at the outset to determine the cholesterol content of the blood along with the fat content but, as will appear obvious from the results, the variations were so extraordinary and so utterly unrelated that latterly this line of investigation was abandoned.

The Chemical and Physiological Properties of Fats.

(1) The chemical and physical properties of fats.

(2) The digestion of fats.

(3) The absorption of fats.

(4) The transport of fats:

- (a) During absorption from the intestine.
- (b) Transport of reserve fat by the blood.
- (c) Transference of fat from blood to organs.

(5) The oxidation of fat.

THE CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF FATS.

(1) The Chemical and Physical Properties of the Fats:

Before proceeding to a discussion of the changes undergone by fats in the animal body it would, perhaps, be advisable to review briefly the more important chemical and physical properties which they possess.

In a strictly chemical sense a fat may be defined as an ester of glycerol and a higher fatty acid, i.e. a long-chained fatty acid, but colloquially a fat is differentiated from an oil which, if it be of animal or vegetable origin, falls in the same chemical group and differs from a "fat" only in possessing a lower melting point.

In chemical physiology, too, the term is often used loosely to include not only the (chemically) true fats, but a number of other substances, many of them containing nitrogen and phosphorus in addition to carbon, hydrogen and oxygen.

Modern practice restricts the term "fat" to the esters of glycerol. Now since glycerol is a trihydric alcohol, it is obviously capable of giving rise to three series of esters in which one, two and three hydroxyl groups have been esterified.

The naturally occurring fats belong almost entirely/

entirely to the third group and contain, therefore, three acidic radicles. Evidently these three groups need not be the same and thus we find that many fats are actually "mixed" esters. It is interesting to note that when two different acid residues are introduced into the molecule the possibility of stereo-isomerism arises. Thus an ester of this type must conform to one of the following formulae:-

I. II.

In the first case, where the two primary alcohol groups have been esterified by the same acid the ester is <u>not</u> optically active; in the second, where the primary groups are attached to different acid groups the middle C. atom of the glycerol chain is asymmetric and the fat may therefore exist in two optically active forms.

Little need be said of the chemical properties of the fats. They are esters and as such are readily hydrolysed, with increased rapidity in presence of dilute acid or alkali, to glycerol and the constituent acid or acids.

A/

A large number of acids has been isolated from naturally occurring fats but a detailed description of them is beyond the scope of this work, for not only would the mere indexing of names occupy many pages, but the great majority of them are of rare occurrence. The commonest are:-

(1) Butyric acid..... $C_3H_7CO.OH$

(2) Palmitic acid ..... $C_{15}H_{31}CO.OH$

(3) Oleic acid..... $C_{17}H_{33}CO.OH$

(4) Stearic acid..... $C_{17}H_{35}CO.OH$

Tributyrin, the ester of glycerol and butyric acid occurs principally in butter fat: tripalmitin occurs largely in vegetable fats; triolein in animal and vegetable oils or low melting fats; and tristearin in animal fats preponderating in the hard fats such as suet. In addition one might mention linoleic acid $C_{17}H_{31}CO.OH$ and linolenic acid $C_{17}H_{29}CO.OH$ which occur in vegetable oils such as linseed oil and confer on them the valuable "drying" properties. Like the vast majority of the acids found in the natural fats these examples, it will be noticed, all contain an <u>even</u> number of C. atoms - a fact the importance of which will be evident when their mode of oxidation in the animal body is considered.

Of the acids mentioned above it will be seen that three - oleic, linoleic and linolenic differ only in the number of hydrogen atoms they contain and/

and that each possesses the same number of carbon atoms as stearic acid. They are unsaturated acids containing one, two and three double bonds respectively. By reduction they may be converted into stearic acid.

Now a natural fat is not usually a single ester but a mixture of two or more (which may themselves be "mixed" esters) and, therefore, it cannot be characterised by the usual criteria of chemical purity. Often, however, fat from a given source is found to contain a moderately constant proportion of unsaturated acid and hence the determination of this proportion becomes important. For this purpose one uses the ability possessed by unsaturated compounds of adding on halogens and in particular, because of the ease of estimation, of iodine. At each double bond two iodine atoms can be added:-

$$-CH=CH- \xrightarrow{I_2} -\overset{I}{\underset{|}{C}}H-\overset{I}{\underset{|}{C}}H-$$

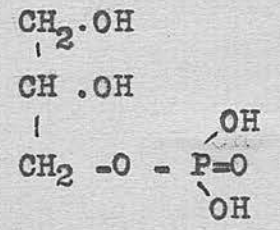
By adding a known amount of iodine to a known amount of fat and estimating the excess iodine by titration with sodium thiosulphate $Na_2S_2O_3$ one can determine the amount of iodine absorbed by the fat. For the sake of convenience it is usual to calculate from this data the "iodine number" of the fat which may be defined as the number of grams of iodine absorbed by 100 grams of the fat. Thus triolein with a molecular/

molecular weight of 884 takes up 6 atoms of iodine i.e. 7769. Hence the iodine number of triolein is:-

$$\frac{776}{884} \times 100 = 86.2$$

The actual separation of the acids liberated by the hydrolysis of fat is a difficult and tedious process but can be accomplished by taking advantage of such factors as their differing volatility in steam; the insolubility of certain hydrazine derivatives; the solubility in ether of the lead salts of saturated acids; the acetyl derivatives of the hydroxy acids and so on.

Closely related to the true fats are the phospholipines or phosphatides. These substances are also esters of glycerol and fatty acids but in them one of the hydroxyl groups of the glycerol has been esterified by phosphoric acid. They contain only two molecules of fatty acid and may be considered as being esters of glycero-phosphoric acid, the formula of which may be represented as:-

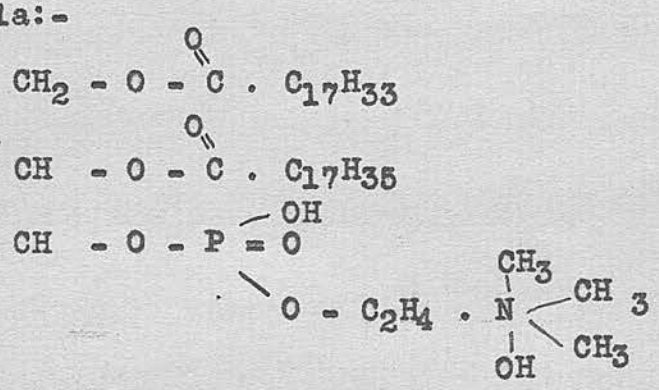


Glycero-phosphoric acid.

In the first place it is to be noted that glycero-phosphoric acid contains an asymmetric carbon atom so that, whether the remaining hydroxyl groups are esterified by the same or different acids, the resulting phospholipine will be capable of existing in optically active forms.

Secondly, since only one hydrogen of the phosphoric acid has been removed the glycero-phosphoric acid is still capable of combining with base and, in fact, the phospholipines on hydrolysis yield not only fatty acid but also a nitrogenous base. In the case of lecithine the base is choline, in cephaline it is β-hydroxy-ethylamine.

Thus lecithine may be represented by the formula:-



Lecithine.

Another group of glycerol esters yields galactose on hydrolysis in addition to fatty acid and glycero-phosphoric acid. These substances are known as galactosides or cerebrosides. They are not well characterised /

characterised and little is know of their exact constitution. It would appear possible, however, that they resemble the phospholipines and that one or both of the hydrogen atoms of the phosphoric acid are esterified by hydroxyl groups of the galactose.

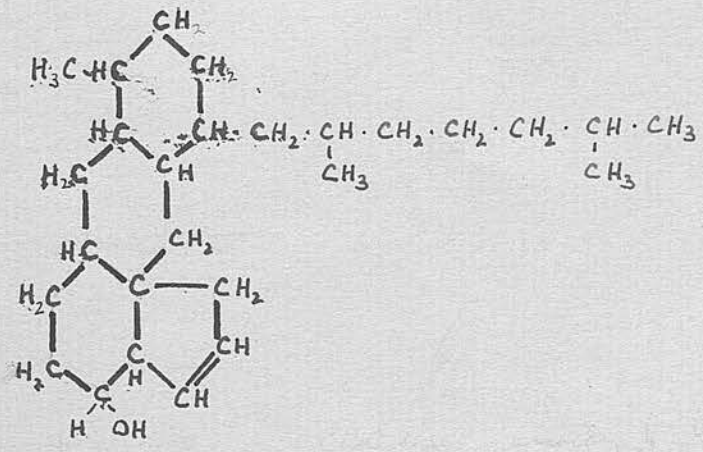
The waxes have been defined as esters of alcohols other than glycerol. While it is difficult to obtain a rigid definition of these substances, this is obviously unsatisfactory since it would include such a substance as ethyl acetate which by no strength of imagination can be considered a wax. Taking into consideration the recognised physical properties of waxes, e.g. appearance - one finds that not only are long-chained acids invariably present but that the alcohols are very complex. Thus Chinese Wax is composed chiefly of ceryl ester of cerotic acid - $C_{27}H_{55}O.OC.C_{26}H_{53}$.

Finally there is a vaguely defined group of lipoids a "dumping-ground" for all fat-like substances which do not fall into any of the above categories or are of undetermined constitution. Among them are cholesterol and the closely related phytosterol, ergosterol, etc.

The sterols are complex unsaturated alcohols, cholesterol, for example having the molecular formula $C_{27}H_{45}OH$ and, probably, the structure may

be/

be represented by the formula:-



Cholesterol, according to Windaus.

Cholesterol has long been known to be very widely distributed in living tissue and to be the principal component of gall-stones. Lately it has sprung into prominence owing to its power, after irradiation, of replacing cod liver oil etc. as a source of fat-soluble vitamin A. Recent work by Rosenheim & Webster (1927), however, has shown that cholesterol itself is not a source of vitamin but that it occurs naturally in association with minute amounts of closely related alcohol (ergosterol) and that it is this alcohol which, under the influence of ultra-violet rays, is converted by a slight chemical modification into the vitamin.

Thus the division of the physiological group of fats may be summarised as in the following table/

	Group Name	Elements present	Chemical Category	Products of Hydrolysis
I.	Fats (and oils) also known as Lipines	C.H.O.	Ester	Glycerol and fatty acid.
II.	Phospholipines also known as Phosphatides	C.H.O.P.N.	"	Glycerophorphoric acid. Fatty acid. N- base (e.g. choline)
III.	Galactosides also known as Galacto lipines or Cerebrosides	C.H.O.P.	"	Glycerophosphoric acid. Fatty acid. Galactose.
IV.	Waxes.	C.H.O.	"	Alcohol other than glycerol. Fatty acid.
V.	Lipoids.	??	Often alcohols	??

(2) /

(2) The digestion of Fat.

(a) In the stomach.

As recently as 1900 it was imagined that fats underwent practically no digestive changes in the stomach until Volhard (1900) found undoubted digestion of the emulsified fat of milk and egg-yolk both by gastric juice obtained after the usual form of test breakfast and also by glycerin extract of the mucosa of the fundus. (In fairness it must be pointed out that Ogata and other observers had remarked that the stomach had a lipase of its own many years ago, but their work received little attention until verified by Volhard).

Since 1900 many observers have confirmed Volhard's findings, some using his own method, others using modifications. Among these workers we may quote Stade (1902), Fromme (1906), Laqueur (1906) and Heinsheimer (1906).

With this considerable evidence of the evidence of the existence of a fat-splitting agent in the stomach the natural question which followed was"From whence did this agent arise?" Apparently there were three possibilities:-

(I) That it was the HCl of the gastric juice.

(II) That a definite gastric lipase was secreted during digestion,

or (III)That the fat splitting agent was arising from/

from the pancreatic enzyme entering the stomach as a result of the reflux of duodenal contents.

It was found that no fat digestion occurred when boiled gastric contents were incubated with egg-yolk emulsion and so it was apparently not the HCl of the gastric juice which exerted the lipolytic action. Volhard also showed that, if the gastric juice contents be strongly acid, then little or no fat-splitting occurred. This is not what one would have expected if the fat-splitting were performed by the HCl.

It has been shown further that fat hydrolysis in the stomach is much decreased if a pure gastric juice is used, while London (1907) working with a Pawlow stomach in dogs (in which reflux of duodenal contents could not occur) was unable to demonstrate lipase in the gastric juice.

These findings suggested that the greater part, if not all, of the fat-splitting originally observed by Volhard was due to reflux of duodenal contents. This view is still widely supported and the presence of a definite gastric lipase regarded as a a myth. Recent evidence, however, points strongly in favour of the presence of a lipase in gastric juice. Hull and Keeton (1917) studied the fat-splitting element present in the gastric juice obtained from Pawlow stomachs and in "normal" stomachs/

stomachs where the pylorus had been ligated to prevent pancreatic regurgitation, secretion being stimulated by gastrin and by food. Their investigations showed that gastric juice really does contain a fat-splitting enzyme of its own. They found it to be sensitive to acid, fifteen minutes exposure to 0.2% HCl being sufficient to destroy it, while if the acidity were neutralised by addition of alkali or mixture with protein, the the lipolytic activity of the enzyme was about five times as great as that of the succus entericus (Hull and Keeton 1917). Thus after a meal and before the acidity of the gastric juice has reached such a concentration as would destroy the lipase (its concentration being kept down by the proteins of the meal), considerable fat-splitting may take place, especially of emulsified fats such as milk.

Other evidence of the existence of gastric lipase was brought forward by Davidsohn (1912) who compared the properties of gastric and of pancreatic lipase and found differences in their optimum reaction. Thus in the case of gastric lipase the optimum H ion concentration for its action is 2×10^{-6} whereas for pancreatic lipase it is 1×10^{-8}. Sodium fluoride was shewn to inhibit the action of pancreatic lipase to a greater extent than in the case of gastric lipase.

There/

There seems little doubt, then, that a lipase is secreted by the stomach but its action is normally small owing to the increasing acidity of the stomach contents and the lack of any means of emulsifying the fat and so exposing a large surface to enxyme action. Lipolytic action in the normal stomach must be of importance only as a means of producing a little fatty acid which, converted to soap immediately on entering the duodenum, is available for the emulsification of fat there.

In pathological conditions, however, where the hydrochloric acid is greatly diminished or absent, fat digestion may occur and be of great value since the absence of HCl will presumably mean an absence of the great stimulus to pancreatic secretion.

(b) <u>In the small intestine.</u>

There is no doubt, however, but that the chief seat of fat hydrolysis is the small intestine due to (I) the fine emulsification which the fat undergoes here and (II) to the presence of fat-splitting enzymes especially that of the pancreas.

<u>Passage into the Small Intestine.</u>

Whether the amount of food fat be large or small, the fat in either case reaches the intestine in small quantities owing to the action of the pyloric valve whose mechanism is influenced by the variations of acidity of foodstuffs undergoing digestion/

digestion. One exception must be mentioned and that is when fat is taken in liquid form (as oil) or suspended in a liquid as milk. In this case it may pass immediately through the stomach like other liquids. The taking of fat in any quantity in the form of oil, however, is unusual.

The fact that such a mechanism exists for the prevention of flooding of the small intestine with fat is important from the point of view of absorption since the small amounts of fats arriving will have a greater opportunity of becoming completely hydrolysed when surrounded by the relatively large amounts of pancreatic and intestinal lipase with which it will come in contact.

This mechanism is upset if excessive amounts of fat are taken into the stomach for, if the inhibition of the gastric secretion be prolonged, the pylorus seems, after a few hours, to lose its tone and allow regurgitation of bile and pancreatic secretion producing considerable fat hydrolysis in the stomach.

It has already been mentioned that emulsification is an important factor in the hydrolysis of fats and it is essential to understand the mechanism by means of which such emulsification is assured.

All food fat contains some free fatty acid and the amount is increased by cooking and also by whatever lipolytic action occurs in the stomach as a result/

result of which, by the time the fat reaches the intestine there is an appreciable amount of free fatty acid delivered along with it. This, uniting with the alkali of the intestinal secretions produces sufficient soaps to emulsify the whole amount and so prepare it for the action of the intestinal lipases, for such emulsification enormously increases the surface area of the fat globules exposed to the action of these enzymes.

The soap film causes a great lowering of surface tension which, combined with the mechanical agitation of the intestinal contents by peristalsis, results in the production of a very fine emulsion.

Bile possesses some power of emulsification but soap$ acting in the same manner is the chief emulsification agent.

There are two fat-splitting enzymes present in the intestinal contents, the chief one being supplied by the pancreatic juice and the other by the intestinal juice.

Boldyreff (1907) found that the secretion collected from a Thiry-Vella fistula in dogs would hydrolyse monobutyrin and also neutral fat and that the action was assisted by the presence of bile. Jansen (1911) has objected to the use of monobutyrin on the grounds of its being split by water alone and the probability of a different ferment - monobutyrinase/

butyrinase (an esterase) being involved.

Lombroso (1912) verified the presence of a fat-splitting enzyme in the secretion of a Vella fistula but found the power of this enzyme to be greatly enhanced in the secretion poured out in response to the introduction of olive oil into the fistula. Thus apparently the introduction of olive oil, i.e. undigested fat, into the small intestine as would occur if the pancreatic secretion were absent, calls forth a secretion of intestinal juice whose fat-splitting powers are greater than normal and whose lipase can thus take on the duties of the absent pancreatic enzyme.

The action of the intestinal juice is, however, meagre when compared with that of the pancreatic juice which exerts a powerful lipolytic action. Claude Bernard was the first to point out such an action finding that a solution of butter in ether rapidly became acid when submitted to the action of the juice.

The following year Berthelot (1851) isolated the fatty acids produced and so determined the nature of the reaction.

Terroine (1910) and Mellanby and Woolley (1914) determined the influence of various factors on the activity of the enzyme working with pancreatic juice. They found that:-

I. /

I. The enzyme acts in neutral solution but the activity rapidly diminishes on the acid side of neutral point while increase in alkalinity increases the activity up to a certain point after which it falls off. The maximum action is observed in an alkalinity of N/150 NaOH (Terroine).

II. Electrolytes increase the action and each salt examined by Terroine (chlorides of K, Mg, Ba, Ca) had its optimum for this effect.

III. Heating the enzyme for 10 minutes at 60° C. causes its destruction.

IV. Addition of bile salts markedly diminishes its stability on heating.

V. It is rapidly destroyed by N/50 HCl.

Thus we see that the mechanism for the digestion of fats in the small intestine is such as to secure almost complete hydrolysis. We have seen lipase to be abundant, being present in gastric, pancreatic and intestinal secretions. That present in the pancreatic secretion alone is sufficient to digest in a short time many times the amount of fat present in the ordinary diet. Under certain conditions mentioned the gastric lipase can digest considerable quantities and even the intestinal lipase can probably effect splitting of the daily fat intake for even in those cases where pancreatic secretion is lacking, very little unsplit fat is found/

found in the faeces, ~~in these cases~~).

Emulsification by soap is an important factor in hydrolysis and there is abundant provision for soap formation made. Finally the continuous absorption removes products of hydrolysis at once providing a clear field for rapid and complete action. Under these circumstances, it is probable that the amount of fat which escapes digestion is negligibly small.

(3) Absorption of Fats.

The original view of the question of fat absorption was what has been called the "Particular Absorption Theory". It assumed that fine particles of emulsified fat in the intestine passed mechanically through the striated border of the epithelial cells and so into their protoplasm. The only modern worker who adheres to this view, it may be added, is Croner (1909). Evidence in its support was brought forward on histological experiments which clearly demonstrated the presence of fine fat globules in the epithelial cells of the intestine after a fatty meal but not during starvation. This was presumed to be the same fat since the fat administered as food had been stained with characteristic stains and the fat globules in the epithelial cells were found to be similarly stained.

This line of argument, however, overlooked the fact that the stains soluble in fat are equally

soluble/

soluble in soap and will remain attached to the soap and be carried with it to the intestinal epithelium so that the presence of similarly stained globules in the protoplasm of these cells is by no means conclusive evidence that such globules have been absorbed unchanged.

Once, however, it was shown that fat hydrolysis in the intestine was almost complete, the particular theory became untenable.

The view of the mechanism of fat absorption accepted at present is founded on the work of Immanuel Munk. Munk (1880) found that fatty acids as well as fats would form an emulsion in the presence of protein provided a little soap were added. Wondering if fatty acids could be absorbed in this form he administered free fatty acid alone to dogs and in collecting the chyle from the thoracic duct was astonished to find that almost the whole of the fat present in the chyle <u>was not fatty acid as he expected but neutral fat</u>. (About 5-10% only of the fat was present as free fatty acid or soap).

This experiment was of vital significance, pointing as it did for the first time to a synthesis of fatty acid into neutral fat during absorption from the intestine. This has been amply confirmed in various ways and is now a well established fact.

It is obvious that a synthesis must have occurred/

occurred between fatty acid and glycerine since only fatty acid was administered - the glycerine for this synthesis is supplied from sources to be described later.

The next question was "Where does this synthesis take place?"

The lymphatic glands lying along the course of the lacteals were suggested, but this theory was effectively disposed of by Moore (1903) who showed that the fat of the chyle collected from lacteals before traversing any lymphatic glands consisted almost entirely of neutral fat and also that the mucous membrane of the intestine, at the height of digestion, contained a mixture of fat and fatty acid, the former preponderating. This work, whilst disrupting the theory of the lymphatic glands as being the seat of chemical change, pointed to the mucous membrane as the site of the actual synthesis.

Munk was fortunate in having a patient - a girl of 18 - "suffering from an elephantiasis of the left leg in which dilated lymphatic vessels were visible under the skin. In the upper third of the leg, below the knee, was a small fistulous opening which discharged clear lymph during fasting periods, but, after a meal containing fat, poured forth a milky fluid. It was on this patient that Munk made/

made most of his classical experiments on fat absorption. Although, on anatomical grounds, it is difficult to understand how the whole of the chyle from the lacteals could be collected from the fistula on the leg, other evidence obtained by Munk pointed strongly to this being the case." (Leathes and Raper (1925). Working with this patient Munk and Rosenstein (1891) proved that this synthesis took place in the human being. They administered erucic acid to this patient and in the chyle only the glyceride erucin was found (erucic acid is a higher member of the oleic series of unsaturated fatty acids). They concluded that the whole of the fat had been hydrolysed in the intestine before absorption but that part of the resulting fatty acid might have been absorbed in the form of a fine emulsion and taken up by the cells of the villi where it was built up into neutral fat. (Similar results to similar experiments have been obtained by other observers (e.g. Bloor 1911, Frank 1898, and Argyris and Frank 1912). This line of reasoning was followed because it was thought that the body could not supply sufficient alkali to convert all the fatty acid resulting from hydrolysis into soap. It was argued that a dog can absorb 200 grams of fat in a day which would require 39.3 gms. of sodium carbonate for conversion into soap. Only some 16 or 17% of this/

this amount of alkali could be provided by the whole blood.

Munk assumed then that "a considerable proportion of the fatty acids produced during digestion is absorbed without previous conversion to soaps and that these fatty acids are taken up in particulate form by the cells of the villi."

Histology, however, has not been able to reveal the presence of stainable fat in the free borders of the epithelial cells although during fat absorption it can readily be demonstrated in the bodies of the cells.

Pflüger (1901-1903) concluded that all fat must be absorbed in solution but Kingsbury (1917) proved that the evidence of his experiments was unsatisfactory.

It must be remembered, however, that fat may be present in cells in considerable amount and yet its presence be undetermined, possibly on account of its high degree of dispersion in the protoplasm rendering it unstainable (Fischer and Hooker, 1916) so that undue stress must not be laid on the histological findings in the investigation of fat absorption.

If, now, there were present in the intestine a solvent for fats or their digestion products, i.e. the fatty acids, then these substances might pass into the cells of the mucosa without giving the staining/

staining reactions of particulate fat or fatty acids. For a long time it has been known that bile would dissolve fatty acids. More recently Moore and Rockwood (1897) and Moore and Parker (1901) have shown that the solubility of a fatty acid or its sodium, calcium or magnesium salt in a solution of bile salts was greatly increased by the presence of lecithine in small amounts. Thus it appears that the presence of lecithine in normal bile magnifies its solvent action on fatty acids and soaps.

Further Kingsbury (1917) showed that bile increased the formation of soaps during the normal periods of digestion by accelerating the reaction between fatty acid and sodium bicarbonate.

Thus we come to the present conception of fat absorption in which it is assumed that the fat is hydrolysed in the intestine to fatty acid and glycerine.

The fatty acid is combined either with alkali to form a water soluble soap or with bile salts to form a compound which is also soluble. The glycerine and the dissolved fatty acids are separately absorbed into the epithelial cells of the intestine in the protoplasm of which, after the fatty acid has been set free from the alkali or bile salt, they are re-synthesised to form neutral fat, the most of which finds its way by the central lacteals into the villi and/

and then by way of the lymphatics to the thoracic duct.

(N.B. In obstruction of the common bile duct a resulting deficiency of fat absorption occurs, but the increase of fat in the stools is due to accumulation of fatty acid and not neutral fat.)

Apart from those of Munk, the most important experiments supporting this modern "chemical theory" are based on the observation of the fate, during absorption and digestion, of substances bearing a strong resemblance to fat either in chemical composition or physical properties. These have usually been given per os and the chyle collected from the thoracic duct or otherwise during the ordinary periods of digestion has been examined to determine whether the fat-like material given as food could be detected in it. The faeces have also been examined to discover the degree of absorption of the substance fed.

Munk and Rosenstein(1891) fed their famous patient with cetyl palmitate and found the collected chyle to contain no cetyl palmitate or cetyl alcohol but the glyceride tripalmitin. This was a definite blow to the Particulate Absorption Theory for, had the food been absorbed in particulate form, then cetyl palmitate should have appeared as such in the chyle/

chyle. It apparently could not be absorbed without previous hydrolysis in the intestine.

Other evidence against the absorption of fat in particulate form has come from experiments with fat-like substances, e.g. paraffin oil, lanoline, etc. These are capable of being converted into fine emulsions but cannot be brought into solution in the intestine.

An emulsion made up of some neutral fats and some hydrocarbon, e.g. albolene, when fed and the faeces examined, shows that all the fat but none of the hydrocarbon has been absorbed. Such an emulsion on microscopic examination shows the particles of neutral fat and hydrocarbon to be exactly the same size and thus forms strong evidence against the mechanical theory of absorption.

Similarly mixtures of fat in the form of lard with vaseline on being fed to rats (Henrique and Hanson (1899 and 1901) resulted in the greater part of the lard being absorbed while 95% of the vaseline was recovered from the intestine.

Connstein (1899) fed 20 grams of lanoline to a dog and recovered almost the whole amount in the faeces. Similar experiments by Bloor (1913) yielded similar results.

It may be concluded then that fatty substances which are insoluble in water (or cannot be changed by digestion into substances (soaps) that are soluble in/

in water, are not absorbed however similar they may be to fat in other particulars.

The conclusiveness of these experiments may be questioned by arguing that some essential physical difference may exist between emulsified fat and emulsified hydrocarbon, ~~and hence~~ In order to prove the case for the chemical theory one should really feed a neutral fat possessing some characteristic that depends on the manner of union existing between fatty acid and glycerol and then to observe whether it appears in an unchanged condition in the thoracic duct or not.

If it appears in an unchanged condition, the fat must have been absorbed through the intestine in an unbroken state (unsaponified) for it is highly improbable that any resynthesis occurring in the intestinal epithelium would result in a recombination of fatty acid and glycerol molecules in exactly the same manner as before.

What is the reason for this primary hydrolysis and secondary resynthesis of fat after its passage into the epithelial cells of the mucosa?

The first apparently obvious reason is that such a mechanism prevents the absorption of fat-like substances which are unsatisfactory for the supply of energy to the body, e.g. petroleum.

Or a similar process may occur as in the case of protein where the protein molecule is completely broken down into "building stones" of amino acids and/

and each tissue builds its own edifice selecting whatever "brick" it requires.

With fats there may be a re-arrangement of fatty acid molecules as a result of which the newly formed fat is more adaptible for use in the organism and approaches more closely the characteristic fat of the animal.

This assumption is supported by experiments based on the observation of differences in the melting points of the administered food fat and resulting faeces (Arnschink, 1889) and a similar variation of food fat and chyle fat in the researches of Munk and Rosenstein(1891).

Frank (1898) working with dogs, and more recently Bloor (1912, 1913, 1914),performed interesting experiments in which the chemical properties of fats before and after absorption were compared by means of melting point, iodine value and mean molecular weight (Bloor)estimations, the melting point representing the solidity of the fat and the iodine value its degree of unsaturation.

Considerable changes were found to occur in these two characteristics before and after absorption-for example, feeding with fat of abnormally low iodine value and high melting point produced a chyle fat of lower melting point and higher iodine value and vice versa. These results could be explained as due to the/

the addition of oleic acid in the first case and addition of some saturated fatty acid in the vice versa case.

In Frank's experiment (1898) a change of melting point occurred in the chyle fatty acids compared with that of the fatty acids of the food fat. Giving ethyl palmitate the chyle fatty acids melted at 50.5° (palmitic acid 63°) and had an iodine value of 32.6, corresponding to an eleic acid content of 36%. The food fat contained no eleic acid so that it must have been added during the resynthesis in the intestinal mucosa or must be normally present in facting chyle.

If a fat consisting mainly of glyceride of saturated fatty acid but with low melting point be given, addition of oleic acid was still found to occur as determined by the iodine value so the change is apparently chemical (and not merely to lower the melting point of the absorbed fat).

Similarly a lowering of the iodine value occurred after feeding with cod liver oil which contains a high percentage of unsaturated fatty acids.

The reason for these changes, some in one direction and some the other, may be accounted for by the difference in absorption rates between the unsaturated fatty acids and the more poorly absorbed higher/

higher fatty acids.

Food fat usually consists of a mixture of fatty acids of varying degrees of saturation and changes in the chyle fat might be expected (Levites 1907).

Bile contains a small amount of fatty acid in the form of neutral fat, lipines or soap which may be absorbed along with the food fat but the amount is insufficient to account for the change in composition which occurs when saturated fats alone are fed.

While this mechanism does not produce a hard and fast standard of fat for distribution to the tissues it, nevertheless, prevents the taking up of a fat differing considerably from the normal connective tissue fat of the animal.

It has been left to Munk and Rosenstein (1891) to perform the only quantitative experiment in which the absorption after a meal containing fat has been followed almost to its completion. Using "lipanin" (olive oil and 6% free oleic acid) which would be almost entirely absorbed, no more than 66% could be accounted for in the chyle. Various natural fats were also used but this was the highest figure recorded. Apart from the quoted anatomical difficulties, it must be remembered that during the period of absorption, there is an increase in the fat content of the blood. This increase is very considerable

at the height of absorption and even without any allowance for immediate utilisation may account for a large proportion of Munk's missing 33%. Doubtless much of this blood fat is absorbed from the lymphatic system but it is possible that some is absorbed direct from the intestine probably directly by the blood capillaries of the small intestine.

This has not been definitely established but Hamburger (1900) ligatured the lacteals and Munk and Friedenthal (1901) ligatured the thoracic duct and found that fat absorption is diminished but not entirely prevented, as would have been the case had the chyle been the only route of absorption.

As to the site of fat absorption, experiments on animals with intestinal fistulae have shown that the small intestine is the main seat of absorption of fat.

No evidence was brought forward of absorption from the stomach and very little absorption occurs after the ileo-colic sphincter.

Greene and Skaer (1914), however, have described fat-like granules appearing in the gastric epithelium after fat meals and indicating absorption by the stomach but Mendel and Baumann (1915) while confirming the above findings failed to find any increase in the fat content of the circulating fluids from the stomach when fat is present in it so that quantitatively this phenomenon is of no importance/

importance in fat assimilation.

Hamburger (1900) reported vigorous fat and soap absorption from the large bowel. As this is somewhat contrary to the usual behaviour of the colon, confirmation of these results is required before overemphasising this point.

(4) Transport of Fat.

A. During absorption from the intestine.

After the fat has penetrated the intestinal mucosa there are apparently two routes it may take. It may, on the one hand, enter the blood capillaries directly, whence it will be conveyed to the various organs via the liver, or, on the other hand, it may travel via the thoracic duct to the great veins at the root of the neck whence it will be distributed equally to all tissues. The avidity with which the liver, under certain circumstances, takes up fat may make the thoracic duct route advantageous when fat is to be laid by in adipose tissue.

Whichever route the fat may take in the first place, ultimately it reaches the blood stream, so that examination of the blood may fairly be expected to yield information regarding the transport of fat. In accordance with this expectation numerous

observers, e.g. Neisser and Braeunig (1907), Neumann (1907) have noted the appearance of minute fat globules in blood drawn from an animal after a meal of fat, the milkiness due to the separation of these globules reaching a maximum 3-5 hours after the meal and disappearing only 12 hours afterwards.

Actual estimations of the blood fat content have amply confirmed this observation (Terroine, 1914). Bloor (1916) has carried out extensive researches with the object of determining the changes in the blood during and following the absorption of fat. He finds, not only that the total fat content of the blood is increased, but that, in particular, the phospholipin content follows a course roughly parallel to that of the total fat, being maximal 4-7 hours after the meal. Moreover, separate analyses of whole blood and plasma showed that most of the new phospholipin was present in the corpuscles. Bloor is inclined to base somewhat far reaching deductions on these results for he considers them to justify the conclusion that most, if not all, of the absorbed fat is removed by the blood cells from the plasma and transformed into "lecithine". In view of the amount of phosphorus available, however, it is doubtful whether very much of the fatty acid is actually converted into phospholipin although the concentration of the latter may become and remain high.

Different/

Different rates of absorption into the tissues of the fat from the plasma and the phospholipin from the corpuscles may account for the continued presence of a relatively small amount of the latter.

Bloor and Knudson (1917) and Knudson (1917) have shown that during fat absorption the cholesterol esters of the blood increase in a manner similar to that observed for phospholipins (though the total amount of cholesterol may change irregularly or remain constant) and that again, the newly formed esters are found principally in the corpuscles. Again, however, it is impossible to decide how much of the fatty acid is actually involved in the increase and whether the cholesteride is passed on to the tissues as such, or is first resolved into its components.

The position is summed up by Leathes and Raper (1925) as follows:- (page 150) - During the absorption of fat from the intestine -

"(1) The amount of fatty acids in different combinations temporarily increases in plasma and corpuscles, rather more, perhaps, in the latter than in the former.

(2) The amount of cholesterol tends to increase up to a certain point but not beyond it, depending, perhaps, upon the amount available in the intestine/

intestine from food or bile; the association of cholesterol with the fat appears thus to be rather accidental than essential to the process either of absorption or of transfer from blood to tissues.

(3) The amount of phospholipine increases, mainly in the corpuscles; the newly-formed phospholipine appears to be made from the absorbed fatty acids, the source of the phosphoric acid and the basic constituents being obscure; it may be reasonably supposed to leave the blood for use in the tissues since the amount in the blood gradually returns to its previous value as the absorption of fat ceases. How much of the absorbed fatty acid is thus dealt with there is no means of saying; the amount may be quite small.

(4) The amount of cholesteride in the blood increases, involving some 10% of the whole amount of cholesterol; the newly-formed ester appears mainly in the corpuscles; whether it is passed on to the tissues as cholesteride, or is merely a temporary combination subsequently reconverted by hydrolysis into free cholesterol and fatty acid, the data cannot decide."

(B) Transport of Reserve Fat by the Blood.

It must be remembered that though the active absorption of fat is undoubtedly accompanied by an increase in the blood fat content, it by no means follows/

follows that an active transportation of fat will necessarily be shown by estimation of the fat in the blood, It may well be that the utilisation may proceed at such a rate that, notwithstanding the increased rate of transport, there is no actual rise in the concentration present in the blood. Normally, it may be supposed that the demand of the tissues for fat remains roughly constant, so that variations in the concentration of fat in the transport medium will be small and irregular. This indeed has actually been found to be the case (Daddi 1898; Mansfield,1910; Terroine,1914). Leathes and Raper (1925) go so far as to state (p. 154) "that it is not possible to follow the migration of fat from the reserve depôts to the working organs by examination of the blood". This, however, is probably an overstatement of the case, for it is not difficult to visualise conditions in which a sudden increase in the general activity of the tissues may call for a greatly increased supply of fat and thence to a definite alteration in the concentration of fat in the blood. The direction and magnitude of the alteration may be different in different circumstances, depending, inter alia, on the amount of fat available in the depôts, the existing concentration in the blood, the avidity of the tissues and the availability of other sources of energy. It seems clear then that although the rate of transportation may so alter with variations in demand

as/

as to keep constant the concentration of fat in the blood, yet at times, with an exceptionally large demand, the call of the tissues may be manifested by a definite alteration in the blood fat. The difficulty of demonstrating the transport of fat from the reserve depôts has been indicated; it remains to outline the evidence which shows that such a transport does actually occur.

The experiments of Zuntz, Rubner, Atwater and others have shown that during starvation a large proportion of the energy required for the maintenance of body temperature and necessary activities is accounted for by the simultaneous disappearance of reserve fat.

Determinations of the respiratory quotient during starvation afford evidence in support of this although their value may, perhaps, be questioned.

Perhaps the best or most convincing evidence in favour of the availability of reserve fat has been obtained from examination of the blood fat during pregnancy and lactation. Engel (1906) found that the fat of the colostrum has the same iodine value as that of the reserve depôts and since Herrmann and Neumann (1912) found a high concentration of fat in the blood just before parturition, it is fair to regard the reserve fat as having been mobilised, in part at least, for the manufacture

of colostrum. Caspari (1899) found, not only that iodised fat when added to the food appeared both in the milk and the fat reserve, but that its secretion in milk continued long after it had been withdrawn from the food and could, therefore, have been derived only from the reserves.

There remains, then, no doubt that the reserve fat can be made available for use by the tissues; equally there can be no doubt that the blood provides the means of transport. A direct proof of this latter fact is afforded by the observation that when fat is mobilised from the reserves at an abnormal rate as in phlorrhizin or phosphorus poisoning, the concentration of fat in the blood is very markedly increased (Lattes, 1911).

(C) Transference of Fat from Blood to Organs.

When abnormal amounts of fat are found in any organ the increase may be due to (1) a decreased rate of oxidation of fats brought in the normal way, (2) to an accelerated importation, or (3) to local synthesis. If such a case can be proved to be due to an accelerated importation from the reserves in adipose tissue there will be provided strong evidence that such an importation takes place, though at a lessened rate, in normal cases.

Indeed/

Indeed such evidence is hardly required for it has been shown that the blood does transport fat from the reserves and this fat must necessarily be passed on to the tissues for use.

It has been shown experimentally that the morbid change of "fatty degeneration " in the liver following phosphorus poisoning is the result of the migration of fat from adipose tissue, since there was no "fatty degeneration" in the liver of the man who had taken phosphorus when he was emaciated by starvation.

Secondly a starving dog was fattened with food containing large amounts of linseed oil. It was then poisoned with phosphorus and the liver was found to contain large amounts of fat which resembled the fat with which the animal had been fattened.

This experiment has been amply confirmed and in addition Rosenfeld (1899) found that after phosphorus poisoning the liver exhibited "fatty degeneration" only in proportion to the amount of fat in reserve.

Abnormal accumulations of fat in the heart and kidney have similarly been shown to be produced by mobilisation of fat from the reserves but, except that the fat is transported by the blood, there is no evidence to show how the transfer is brought about.

Even/

Even in the case of fat absorbed from the intestine, a case in which the transport by the blood can be followed quantitatively, we are unable to indicate the mechanism by which it passes from the blood to the tissues.

(5) The Oxidation of Fat.

The changes in the fat deposited in the liver.

The variation in the iodine value, indicating as it does the degree to which fatty acid is unsaturated, brings forward evidence that the liver has the power of desaturating fat since the iodine value of the liver fat is much greater than that of the fat fed.

Desaturation consists in the insertion of double bonds in the fatty acid chain - a weak point in the chain where it is liable to break on oxidation. The resulting fragments may then undergo further oxidation in the same way as the original molecule.

This desaturating power of the liver may, however, be a preliminary part of the rôle of the liver in the metabolism of fats - a mere preparatory reaction for the building-up of fatty acid radicles into the complex molecule of lecithin, which is known to contain highly unsaturated fatty acid radicles.

The extraction of fats by alcohol-ether from normal and pathological livers shows that the lecithins which (precipitated by acetone) have higher iodine values (i.e. are more unsaturated) than the neutral/

neutral fats extracted from the same liver, which neutral fats, in their turn, have a higher iodine value than the depôt fat of the same animal.

It is interesting to note in passing that when the liver contains a good store of glycogen there is little or no fat present and vice versa.

The fat in the tissues is very different from that found in the liver or depôts. Only 60% of this fat consists of fatty acid which is present chiefly as part of the lecithin molecule and accounts for the high iodine value. Some is present, probably as simple glyceride in a highly unsaturated and therefore very fragile condition.

The Production of Fatty Acid from Carbohydrate.

$CH_2OH - (CHOH)_4 - CHO$ ..... $CH_3 - (CH_2)_4 - COOH$

Glucose ..... Fatty acid (palmitic)

The conversion necessary in the above transformation will obviously entail:

(1) A considerable alteration in the structure of the molecule,
(2) The removal of oxygen,
(3) The fusion of several glucose molecules into one molecule of fatty acid.

That is to say, the process of conversion of carbohydrate into fat is one of reduction and the resulting molecule must be capable of releasing more energy when it is oxidised than the original molecule of carbohydrate.

It/

It is obvious that the system of O_2 - CH_2 (which corresponds to fat) will yield more energy on oxidation than that of O_2 - CHO (which corresponds to carbohydrate). This explains why one gram of fat yields 9.3 calories of heat while one gram of carbohydrate yields only 4:1.

Fatty acid, therefore, contains more potential energy than sugar and in explaining its synthesis from sugar in the animal body, the source of the extra energy must be indicated. This is dependent on the oxidation of some sugar molecules (which do not themselves become changed to fatty acid) proceeding side by side with the reduction which affects the others and is represented in the outcome of the reaction by the combustion products of CO_2 and H_2O.

$$6\ C_6H_{12}O_6 + 13\ O_2 = 20\ CO_2 + C_{16}H_{32}O_2 + 20\ H_2O$$

glucose (fatty acid)

Normally the intake of oxygen compared with the output of CO_2 in respired air shows a slight deficit of CO_2 and thus the respiratory quotient (R.Q. = CO_2/O_2 is usually less than unity. During the extensive conversion of carbohydrate to fat, seen during the autumn in hibernating animals, the R.Q. rises up to 1.4 in many cases. That is to say, there is excess of CO_2 output over O_2 intake just as the above/

above equation shows.

The entire dissimilarity in chemical structure of fat and carbohydrate suggests that a primary step in the conversion must be through the breakdown of the C. chain into comparatively simple molecules from which the fat molecules are then reconstructed and the unnecessary oxygen set free.

The Method by which Fatty Acid is broken down.

In the laboratory ordinary oxidising reagents attack the fatty acid chain at the alpha C - atom (i.e. the one next to the carboxyl (COOH) group). This does not occur in the animal body, however, Such a reaction, if it took place, would leave behind a smaller chain with an uneven number of C. atoms and such chains are never found in animal fats. Indeed all the commoner fats contain, as we have seen already, an even number of C. atoms:-

Butyric .... $C_4H_8O_2$

Palmitic.... $C_{16}H_{32}O_2$

Stearic..... $C_{18}H_{36}O_2$

Oleic..... $C_{18}H_{34}O_2$

The intermediary substances which are produced during the gradual breakdown of the fatty acid molecule are of such a transitory nature that it is impossible for them to accumulate in sufficient amount/

amount to permit of isolation, or even detection, by chemical means.

When anything occurs to upset fat metabolism, however, as in diabetes mellitus, where the tissues are deprived of carbohydrates, the oxidation of fat stops short at the 4 C-atom stage of butyric. This seems to form a residue more resistant to oxidation than the remainder of the fatty acid molecule. Thus, instead of readily undergoing further oxidation to CO_2 and H_2O as under normal conditions, it becomes only partly oxidised to β-oxybutyric acid, which may become further oxidised to aceto-acetic acid and then decomposed to acetone.

These substances are definitely harmful, giving rise to the symptoms of acidosis or ketosis of varying severity.

This was the first inkling obtained of the intermediary metabolism of fats, i.e. in the pathological conditions and it seemed scarcely logical to assume that the same processes would be at work under normal conditions.

Knoop (1905), however, performed experiments which would seem to indicate that such is the case. He thought of introducing into the fatty acid molecule some group which is resistant to oxidation in the animal body so that, when such cleavage product was reached, evidence would be obtainable by chemical means. The phenyl (C_6H_5) group was found/

found to have this effect. By feeding an animal with phenyl derivatives of acetic, propionic, butyric and valeric acids it was found that the urine contained either hippuric or phenaceturic acid. Both of these are compounds of aromatic acids with glycocoll or amino-acetic acid (one of the protein "building stones" always available in the organism to form such compounds).

Thus:-

(1) $C_6H_5COOH + CH_2NH_2COOH = C_6H_5CONHCH_2COOH$

(Benzoic acid) (glycocoll) (hippuric acid)

(2) $C_6H_5CH_2COOH + CH_2NH_2COOH = C_6H_5CH_2CONHCH_2COOH$

(phenylacetic acid) (glycocoll) (phenaceturic acid)

When either benzoic acid or phenylacetic acid is found in the body as a result of the oxidation of the phenyl derivatives of the higher fatty acids the acid combines with glycocoll as above. From this it follows that if oxidation occurs so that 2 carbon atoms are thrown off at a time (β-oxidation) fatty acids with an even C-chain should yield phenaceturic acid and those with an uneven chain hippuric acid. This was found to be the case. Emden's experiments with defibrinated blood perfusing freshly excised liver are strong supporting evidence in favour of this theory.

Chemists were rather puzzled as to the mechanism/

mechanism of such a process of oxidation when in the laboratory oxidation occurred always at the alpha - C. atom with ordinary oxidising agents.

Dakin (1922) has thrown light recently on these difficulties, however, by showing that hydrogen peroxide (H_2O_2) oxidises fatty acids in this manner.

Thus normal saturated fatty acids and their phenyl derivatives can undergo oxidation, not only in the animal body but also in vitro, in such a manner that the two (or some multiple of two) terminal C-atoms are removed at each successive step in their decomposition.

Evidence in support of Knoop's β-oxidation theory.

Emden's experiments, referred to briefly in the preceding section, showed that the small quantity of acetone, formed on perfusing a freshly excised liver with defibrinated blood, was greatly increased when certain amino-acids were added to the blood prior to perfusion.

Further investigation showed that, of the normal fatty acids from butyric to decoic acid, all those and only those, with an even number of C. atoms gave rise to marked increase in acetone formation. Further experiments showed that the acetone was derived from decomposition of aceto-acetic acid.

This/

This supported the contention of Knoop regarding the oxidation of the fatty acid molecule by loss of 2 C-atoms at a time, i.e. by β-oxidation.

This assumption furnishes a satisfactory explanation of the widely differing behaviour of the fatty acids with an even and with an uneven number of C-atoms as regards their ability to form aceto-acetic acid.

The oxidation of a normal fatty acid with an even number of C-atoms would lead to a straight 4 C.chain from which aceto-acetic acid would be formed, whereas fatty acids with an uneven number of C-atoms would yield chains of either 3 or 5 C-atoms which, for structural reasons, would not yield aceto-acetic acid.

Normally, of course, aceto-acetic acid is not the end-product of fat metabolism but is itself an intermediate oxidation product undergoing further oxidation to $CO_2$and H_2O.

It is merely fortunate for experimental purposes that it appears to be more stable than most other intermediate products of fat disintegration.

Another important fact is that it is readily converted into acetone which may be distilled off and easily estimated quantitatively.

Formation/

Formation of Aceto-Acetic Acid from Fatty Acids in Diabetic Organisms.

Administration of the salts of butyric and iso-valeric acid led (in the experiments of Baer and Blum) to an increased excretion of β-hydroxy-butyric acid, aceto-acetic acid and acetone, while propionic acid and normal valeric acid did not.

The more recent feeding of diabetic patients with intarvin (a fat with an uneven number of C-atoms) bears out this principle, but clinical results of such treatment are not so spectacular as theory would have led one to believe. (For further details the reader is referred to Dakin's monograph on "Oxidations and Reductions in the Animal Body").

EXPERIMENTAL SECTION

(A) A brief outline of the chief methods of investigation employed.

(i) Estimation of Blood Fat.

(ii) Estimation of Blood Cholesterol.

(iii) Estimation of Blood Sugar:

(a) Benedict's Method.
(b) Folin and Wu's Method.

(B) Detailed account of the estimation of blood fat.

(i) The apparatus (with diagrams)

(ii) The solutions.

(iii) The Technique.

(C) The efficiency of the technique.

EXPERIMENTAL SECTION

(A) A Brief Outline of the Chief Methods of Investigation employed.

Methods of Estimation:

Although the main subject of investigation has been the blood fat content, it has been found essential as the work expanded to supplement this line of investigation with others. In this section then will be found a brief outline of the methods of investigation used throughout the work and in the following section a more detailed account of the estimation of fat content of blood.

(i) Estimation of Blood Fat.

Stewart & White's Method (1925).

(a) 2 cc. of whole blood added with constant shaking to alcohol-ether mixture in a 50 cc. flask (graduated round bottomed flask).

(b) Boil mixture in water bath, cool, make up to 50 cc. with alcohol-ether.

(c) Mix thoroughly and allow to settle.

(d) 25 cc. of clear super-natant liquid withdrawn for analysis and transferred to 50 cc. conical flask.

(e) Drive off the contained ether by evaporation in steam oven.

(f) Add 5 cc. N/10 NaOH and 5 cc. absolute alcohol and/

and allow to evaporate almost to dryness in steam oven (takes about two hours).

(g) Add 5 cc. N/10 HCl with glass beads (fatty acids liberated).

(h) Boil down to 1 cc. (over bunsen) to drive off absorbed CO_2.

(i) Residual liquid transferred quantitatively to 10 cc. volumetric flask. Absolute alcohol used for washings and for making volume up to 10 cc.

(j) 1 cc. of (i), that is, one-tenth of total fat, titrated against N/10 NaOH from Rehberg burette using 0.2 cc. phenolphthalein (alcoholic solution 0.1%) as indicator.

Thus it is easy to see that starting with 2 cc. of whole blood, at stage (d) above we are dealing with the total fat extracted from 1 cc. blood, for we take only 25 cc. of the total volume of 50 cc.

At (j) we are taking a tenth of this amount for analysis and so the result of the titration will represent fatty acid neutralised in $\frac{1}{10}$ cc. of blood.

The correction derived from macro and micro-titration of the acid and alkali used with a standard amount of titration fluid (1 cc.) and a standard amount of indicator (0.2 cc.) was 0.002.

Thus, for example, if the reading on the Rehberg burette told us that 0.0247 cc. of N/10 NaOH were required to neutralise the sample under investigation, then the reading, corrected for indicator error, would be

$$0.0247 - 0.002 = 0.0227$$

The custom followed throughout these experiments is to/

to calculate the fat as tristearin and since 0.10 cc. of N/10 NaOH is equivalent to 29.6 mg. of tristearin we say:-

0.0227 x 29.6 = amount of fat present in $\frac{1}{10}$ cc. whole blood expressed in mgs.

It is usual, however, to express these things in terms of milligrams per 100 cc. of whole blood.

Therefore

Amount of fat in 100 cc. whole blood = 0.0227 x 29.6 x 10 x 100

= 671.92.

(ii) Estimation of Cholesterol Content.

(Bloor's Modification (1915) of the Autenrieth-Fund process (1913)).

In this estimation the required alcohol-ether extract of blood can be withdrawn (with care) from the remaining 25 cc. of alcohol-ether extract used in the previous fat estimation. It must be withdrawn at once, however, otherwise evaporation will upset the results.

Method:

Take 10 cc. of alcohol-ether extract (as above) and run into a 50 cc. conical flask. Evaporate down almost to dryness in the steam oven and decant with pure chloroform taking about 3-4 cc. each time and boiling down on steam bath to half the quantity and tipping the residue into a 10 cc. cylinder. After three decantations allow the contents of the cylinder to cool and then make up to 5 cc. with pure alcohol/

alcohol.

Now take the standard solution of cholesterol (representing in these experiments 0.4 mgms. of cholesterol) and put 1 cc. of the solution into a 10 cc. cylinder and add pure chloroform up to 5 cc. To each cylinder now add 2 cc. of anhydrous acetic acid and 0.1 cc. of pure sulphuric acid and mix thoroughly and put away in a dark cupboard for 15 minutes. At the end of this time the solutions will have changed into varying shades of green which are now compared against the standard by means of the colorimeter.

Set the fields of the colorimeter so that each half is equally illuminated. Place the standard solution in the right-hand cup and fix it at some suitable reading where a good colour is obtained. (20 has been found most usually suitable). The unknown solution is placed in the left hand cup and its colour is matched with the standard and the reading on its scale noted. It is usual to take six such readings and to take the mean of them.

For example: In our experiment 10 cc. of alcohol-ether extract were taken, i.e. $\frac{1}{5}$ of 2 cc. of blood, i.e. $\frac{2}{5}$ cc. blood since the original extract was made with 2 cc. of whole blood diluted up to 50 cc. with alcohol-ether mixture. We will suppose the final mean reading for the solution was 12.3 mm. Now the/

the standard was at 20 mm. and represented 0.4 mgms. of cholesterol. If the unknown matched the known standard at 1 mm. then it would obviously be 20 x as strong as the standard, i.e. it would contain 20 x 0.4 mgms. cholesterol. But it matched the standard at 12.3 mm. Therefore mgms. cholesterol represented = $\frac{20 \times 0.4}{12.3}$ = .650

But this is with $\frac{2}{5}$ cc. of blood and, as we mentioned before, these things are expressed as so much per 100 cc. blood.

So:-

$\frac{2}{5}$ cc. blood contain 0.65 mgms. cholesterol.

Therefore 100 cc. blood contain $\frac{5}{2}$ x .65 x 100 mgms. cholesterol

= 162.5 mgms. cholesterol

(iii) Estimation of Blood Sugar Content.

In the earlier part of the research the method of estimation was that of Benedict. It was found, however, that the method gave results which were consistently high when compared with those obtained by means of the Folin and Wu technique. Subsequently the Folin and Wu method alone was used and was found more satisfactory.

(a) The Benedict method (1918).

Take two 2 cc. of blood (immediately after shaking up in oxalate. The volume of blood withdrawn was usually about 6-8 cc. as it was required to estimate/

estimate both both fat and sugar contents in each sample. It was of vital importance that no unnecessary delay occurred in "fixing" the sugar content in acid) and place in a 25 cc. graduated flask. Add 5 cc. of distillled water to lake. When laking has occurred make up to volume with picric-picrate mixture. Shake up and allow to stand five minutes.

Filter the contents into a test tube graduated at 8 and 12½ cc. and allow the filtrate to reach the 8 cc. mark. Now add 2 cc. of accurate 10% solution of anhydrous Na_2CO_3. Dilute to 12½ cc. with distilled water and shake up. Immerse in a water bath for 10 minutes. Cool rapidly under the tap and compare on the colorimeter against Benedict's Standard.

Set the standard at 20 and let us assume the colour to be matched when the unknown is at 8.

Then S(standard) representing 0.64 mgms. of glucose = 20

U (unknown) " $\frac{8}{25}$ of glucose in 2 cc. of blood ——— = 8

$\therefore \quad S/U = .64/.64 = 20/8 = 2.5$

$\therefore$ Mgms. present in 100 cc. of blood = 2.5 x 100

= 250 mgms.

(b) <u>The Folin and Wu Method.</u>

<u>Sugar reagent</u>. 35 grms. molydic acid
5 grms. sodium tungstate
Add 200 cc. of 10% NaOH and 200 cc. water
Boil vigorously for 20-40 minutes.
Cool, dilute to about 350 cc. and add 125 cc. concentrated (85%) phosphoric acid. Dilute to 500 cc.

Alkaline copper solution.

Dissolve 40 grms. of pure anhydrous sodium carbonate in about 400 cc. of water. Add 7.5 grms. of tartaric acid and, when it has dissolved, 4.5 grms. of crystallised copper sulphate. Mix and make up to a litre. If a sediment forms, decant the clear liquid.

To test for the absence of cuprous copper from the solution transfer 2 cc. to a test tube and add 2 cc. of the molydate-phosphate solution. The dark blue colour should almost completely disappear.

Standard Sugar Solutions.

Preservative solution: 2.5 grms. of benzoic acid dissolved in a litre of boiling water. Dissolve the required quantity of pure glucose in 50 cc. of benzoic acid solution and make up to 100 cc. with water.

Take 2 cc. of freshly oxalated blood and place in 25 cc. graduated flask, washing with distilled water, 2.5 cc. of sodium tungstate (10%) and 2.5 to 3 cc. of sulphuric acid ($\frac{2}{3}$ N). Make up to volume with distilled water, shake and filter into conical flasks.

Determination: Transfer 2 cc. of the tungstic acid blood filtrate (obtained exactly as in the Benedict technique using the tungstic acid solution in place of the picric mixture) to a blood sugar test tube and, to two other similar test tubes (graduated at 25 cc.), add 2 cc. of standard sugar solutions containing respectively 0.2 and 0.4 grms. of dextrose.

To each tube add 2 cc. of the alkaline copper mixture. The surface of the mixture must now have reached the constricted part of the tube; if the bulb is too large for the volume (4 cc.) a little (not more than 0.5 cc.) of a diluted (1:1) alkaline copper solution may be added. Careful selection of tubes with bulbs of correct volume will avoid any difficulty, however.

Transfer the tubes to boiling water and heat for exactly 6 minutes. Transfer to cold water and allow to cool, without shaking, for 2-3 minutes.

Now add to each tube 2 cc. of the molybdate phosphate solution. When the cuprous chloride is dissolved, dilute to the 25 cc. mark with distilled water, insert a rubber stopper and mix thoroughly. Make the reading in the colorimeter as in the Benedict technique.

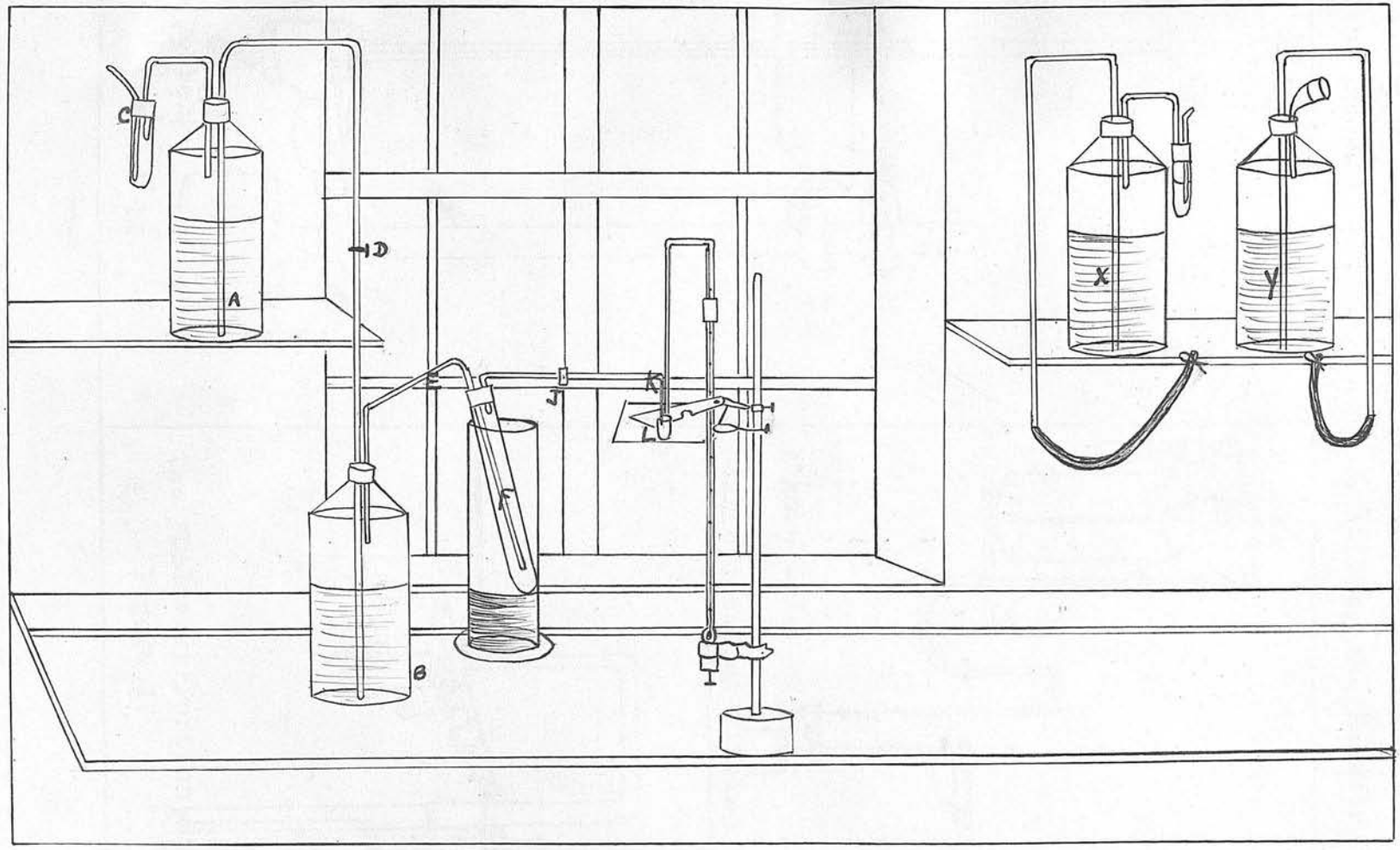
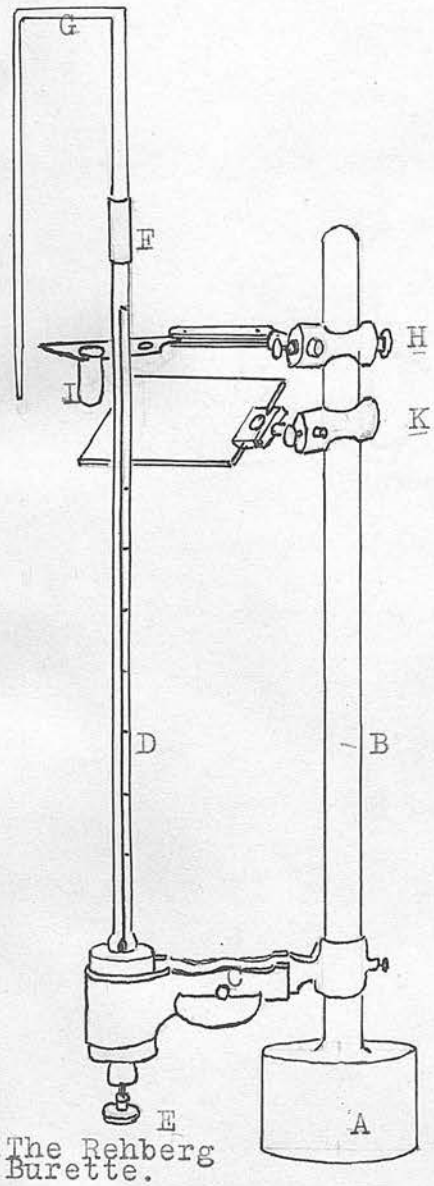


Figure 1.

The Rehberg Burette



The Rehberg Burette.

Figure II.

Filling the Burette

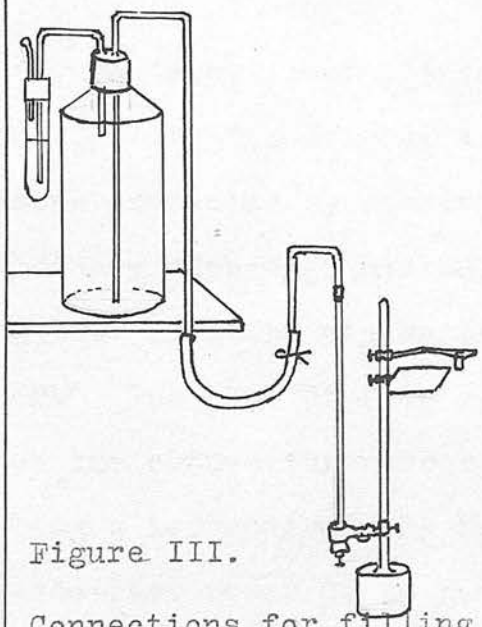
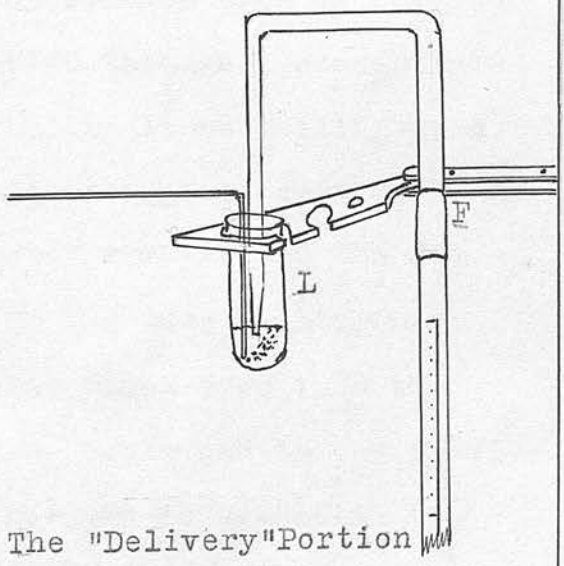


Figure III.

Connections for filling burette.



The "Delivery"Portion

Figure IV.

(B) Detailed Account of the Estimation of Blood Fat.

(i) The Apparatus.

The accompanying figure (Fig. 1) simplifies the description. At the left of the diagram A and B represent two winchesters connected by rubber tubing and forming an ordinary siphonage system, the water from A flowing over into the winchester B and displacing air from B. The rate of flow is regulated by the tap D on the connecting rubber tubing and the air entering A is rendered CO_2 free by passing through the soda-lime tower C. (a really unnecessary precaution as will be evident later). The displaced air from the winchester B is carried along the tube E and bubbled through a concentrated soda solution contained in the large boiling-tube which rests on cotton-wool in a glass cylinder. Any trace of CO_2 is consequently removed and the CO_2 free air is now carried by the tube H into the capillary tube K which dips right down into the fluid undergoing titration, contained in the small test tube L. Such a procedure is essential to ensure thorough mixing of the titrating fluids which are so small in amount as to necessitate the constant flow of a stream of CO_2 free air bubbles through them to enable the determination of a definite/

definite end point. If this procedure is not adopted the demarcation of the end point becomes impossible as the colour falls to the bottom of the small test tube and lies there instead of being diffused throughout the fluid. This small test tube is part of the Rehberg burette which will be described separately.

The Rehberg Burette (Rehberg 1925).

(See accompanying diagram, Fig. 2)

This instrument consists of a metal standard with solid base A and upright B. The bracket C supports the burette D which is a graduated capillary tube with a bulb at its base containing mercury. The lower limit of this bulb is sealed by a metal cap held in position by sealing wax through the centre of which passes a fine threaded screw E. Moving this screw anti-clockwise results in the entrance of the point of the screw into the bulb of the tube and a consequent forcing up of the contained mercury into the graduated capillary portion of the tube. This portion of the tube is graduated from below upwards into 100 divisions. The whole graduation constitutes 0.1 cc. and thus it follows that each division represents 0.001 cc.

Attached to this portion of the burette (see Fig. 4) by means of a short, thick junction of rubber tubing F is the delivery portion of the burette - a double/

double right-angled piece of capillary tubing tapering to a point which should come just below the surface of the fluid undergoing titration in the small test tube L. The point of this delivery tube is lowered into the test tube or taken out of it, as the case may be, by lowering or raising the bracket C on the upright B, the screw with which the bracket is supplied allowing one to clamp the bracket at any point.

Filling the Burette.

This whole capillary system is filled with N/10 NaOH solution by the following method. (See Fig. III).

The delivery portion, along with the thick rubber joint F, is removed and the tapered point of the tube inserted into the rubber tubing from the winchester containing the CO_2 free N/10 NaOH.

The mercury is now forced up the remaining portion of the capillary system by turning the screw E anti-clockwise until the very top of the tube is reached.

By releasing the clips on the tubing connecting the "delivery Tube" with the N/10 NaOH winchester, soda solution runs through the delivery portion which, when completely filled (and still overflowing so as to avoid air-gaps) is rapidly attached to the burette in its original position. We have now got the delivery portion of the burette filled with decinormal soda solution while the remainder/

remainder of the burette is completely filled with mercury. The attachment to the winchester containing the soda solution is still maintained and the screw turned, this time in the reverse direction. This results in the mercury falling and as it falls it draws in the N/10 NaOH after it. When the mercury is passed the zero mark at the bottom of the burette the tube connection with the N/10 NaOH winchester is removed and the mercury moved up to the zero mark. The tip of the delivery tube is now wiped with filter paper to remove any excess of soda solution which may be outside the tube or at its tip and the burette is now ready for use.

Another screw H (Fig. 2) supports the bracket in which the test tube L is supported. This has a movable arm and sockets for three test tubes as can be seen in the diagram.

A final screw K (Fig. 2) supports a porcelain reflector which can be clamped in any position desired.

One cc. of the "unknown" fluid is placed in the test tube L along with 0.2 cc. of 0.1% phenolphthalein as indicator. The point of the delivery tube is lowered into the test tube until the point dips just below the surface of the fluid. The capillary tube leading the CO_2 free air is now placed into the test tube so that its end dips right to the bottom of the fluid. The tap J (see

Figure/

Figure I.) is now turned on and regulated so as to secure a steady current of bubbles which ensures thorough mixing. The screw E of the Rehberg burette is now carefully turned anti-clockwise (thus forcing the soda solution out of the delivery tube) and the test tube L watched for the first occurrence of a diffuse pink colour.

Immediately this appears the burette is lifted clear of the solution (by raising the clamp C, Figure II) and the level of the mercury read off. If the reading is 27, we know that 0.027 cc. of N/10 NaOH have been required to neutralise the acid in the"unknown" fluid

The main object of the Rehberg burette is to facilitate the estimations of small amounts. The ordinary burette is limited in its usefulness in accurate work in so far as the smallest drop one can produce is greater than 0.01 cc. The Rehberg burette overcomes this disadvantage with ease.

Pipettes. It is important to note that throughout the whole series of experiments the same accurately standardised pipettes were used:-

one 25 cc. pipette.
" 5 cc. "
" 2 cc. "
" 1 cc. "
" 0.2 cc. "

Flasks. One dozen 50 cc. volumetric flasks.
" " 50 cc. conical flasks.
" " 10 cc. volumetric flasks.

In/

In addition two winchesters X (containing N/10 NaOH) and Y (containing N/10 HCl) (see Fig. 1) were fitted with right-angled tubing to the end of which was attached rubber tubing with clip. The fluid passes over by siphon action and the pipettes are filled by connecting them up with the rubber tubing and releasing the clip. The air entry into each winchester is guarded by a soda-lime tower and the winchester of N/10 NaOH is waxed. By these means the NaOH solution is obtained with a minimum of contamination by atmospheric air. Similar precautions are scarcely necessary with the decinormal acid solution contained in the winchester Y but were nevertheless taken.

Finally an artificial daylight electric bulb was used as the source of light for all estimations. In the earlier experiments it was noticed that the end-point varied in different lights, e.g. ordinary electric light and daylight. This source of error was overcome by viewing the end-point with this "daylight" bulb as the constant source of illumination.

(ii) Solutions.

(a) CO_2 free decinormal NaOH solution kept in paraffined winchester with a soda-lime tower guarding the air inlet. The required amount of solution was syphoned over through tubing guarded by stop-cock and/

and clip and so the burette or pipettes were filled without the solution coming into contact with air at all.

It is essential that the standardisation should be most accurate and absolutely identical with the standardisation of:-

(b) decinormal hydrochloric acid solution which is similarly housed in a winchester and withdrawn by means of siphonage.

(c) Anhydrous spirit also guarded by means of soda-lime tower. All this anhydrous spirit had to be re-distilled with caustic soda stick in order to over-come the variability of acidity which the spirit showed unless this precaution were taken.

(d) Alcohol-ether mixture containing one part of ether to three parts of alcohol (re-distilled anhydrous spirit used).

(e) Ether. The type used was always ether. sulph. pur. .720.

(f) Concentrated NaOH solution for the CO_2 trap already described.

(g) Solution of neutral tripalmitin with 0.3 grams of tripalmitin in 50 cc. of ether.

(h) Solution of neutral tripalmitin with 0.6 grams of tripalmitin in 50 cc. of ether.

(j) Acid potassium phthalate solution N/100 and N/10 for standardising solutions.

(k) Alcoholic solution of phenolphthalein of 0.1% strength as indicator.

(iii) Technique.

About 5 cc. of blood are withdrawn from the patient's median basilic vein by means of a record syringe and transferred at once to a test tube containing a little oxalate with which it is thoroughly mixed at once, any delay or incomplete mixing leading invariably to clotting which necessitates rejection of the specimen.

2.0 cc. of this oxalated blood is added with constant shaking to about 20-25 cc. of the alcohol-ether mixture in a 50 cc. volumetric flask. This mixture is brought just to boiling point (on the steam bath), thus precipitating the proteins, and is then allowed to cool. When cool the volume is made up to 50 cc. with alcohol-ether mixture and thoroughly mixed after which it is allowed to settle. The flask should be corked to prevent evaporation.

25 cc. of the clear supernatant fluid are now withdrawn and transferred to a 50 cc. conical flask. This flask is now transferred to the steam oven, and when the ether has evaporated off (the flasks are usually left in the oven for about 15 minutes) 5 cc. of N/10 NaOH and 5 cc. of anhydrous spirit are added. (The transference of the NaOH is performed without the fluid coming into contact with air as already described) and the mixture replaced in the steam oven and left until almost evaporated to dryness. This process usually takes about two hours. 5 cc. of/

of N/10 HCl is now added to the mixture which exactly neutralises the whole of the N/10 NaOH and liberates the fatty acids produced during hydrolysis. This mixture is now boiled down (over a bunsen flame, guarded with gauze) to about 1 cc. Glass beads should be placed in the solution for this process.

In this way the CO_2, which is inevitably absorbed during manipulations, is driven off. The residual liquid is now transferred quantitatively to a 10 cc. volumetric flask, absolute alcohol being used for the washings and for making up the volume to 10 cc.

1 cc. of this final solution is now titrated with N/10 NaOH from the Rehberg burette in the manner already described. It will be noted that, starting with 2.0 cc. of blood we took only 25 cc. of the alcohol-ether mixture, i.e. the amount of fat contained in 1 cc. of blood. This final titration in which we used only $\frac{1}{10}$ of the amount of fat originally taken is then equivalent to the amount of fat in $\frac{1}{10}$ cc. of blood.

Practical Points in the Manipulations.

The withdrawal of blood:

(1) Put the tourniquet on really tightly and select a really prominent vein.

(2) Thoroughly mix the oxalate and blood by good, hard shaking. Gentle "swinging" results invariably in clotting before one has cleaned the syringe.

(3) /

(3) Clean the syringe and needle at once.

(4) All pipettes used in measuring blood samples should be run through cold water at once when the blood adhering to the sides is readily removed. Otherwise, delay causes considerable inconvenience.

The flasks, etc.

These must all be thoroughly clean. Any flask after having been in cleaning fluid (H_2SO_4 and potassium ferrocyanide) must be thoroughly rinsed with tap water. When it is imagined that the flask should contain no further trace of acid, it should be rinsed again three times with tap water, then three times with distilled water and finally rinsed with anhydrous spirit and dried in the steam oven.

Strict attention to these details is of the utmost importance as the slightest trace of acid in one of the flasks would throw the calculation hopelessly out since the result depends on the neutralisation of acid by alkali.

The same routine is adopted with pipettes except that instead of the final drying in the oven the pipette is attached to a suction pump and thus dried by a current of air.

The boiling of the alcohol-ether-blood mixture in the first stage.

Bumping and spurting with disastrous loss of fluid is so liable to occur that the following warning is necessary. Place the flasks on the steam bath with the steam turned off and gradually turn/

turn the steam on till boiling occurs.

Driving of the absorbed CO_2 by boiling over a bunsen.

This must be done with constant shaking to prevent spurting. The flask should be held by a clamp.

It is advisable to waste as little time as possible in the manipulations after the addition of the N/10 HCl but to proceed at once to the titration obviating, as much as possible, any exposure to atmosphere.

(C) The Efficiency of the Technique.

Hitherto the small amounts of fatty acid obtainable from the 2 cc. of blood usually available for routine examinations has rendered it impossible to utilise, with any degree of accuracy, the hydrolytic method of estimation in which the fat is saponified by sodium hydroxide and the excess alkali titrated with acid.

The micro-burette of Rehberg, however, delivers 0.1 cc. of solution and can be used for titrations requiring fractions of that amount with at least as great accuracy as is attainable with the 10.0 cc. of macro-titration.

Since 0.10 cc. of N/10 NaOH is equivalent to 26.86 mg. of tripalmitin or 29.67 mg. of tristearin, the burette can evidently be used to titrate the fatty acids liberated by hydrolysis from the fat contained in only 1.0 cc. of normal blood.

It was attempted to estimate known amounts of synthetic tripalmitin. A solution of 0.6 gms. of neutral tripalmitin in 50 cc. of ether sulph. pur. .720 was made and six volumetric flasks (50 cc.) containing a little alcohol-ether extract, were prepared. Into two of these (flasks 1 and 1a) was placed 0.5 cc. of the above solution (i.e. 6 mgms. of fat) into two other flasks (2 and 2a) 1 cc. was added and into flasks 3 and 3a 2 cc. of fat solution was placed. These flasks were now transferred to the/

the steam bath and brought to the boil, cooled and the volume made up to 50 cc. with alcohol-ether mixture. 25 cc. of this solution was now transferred to 50 cc. conical flasks. Although these steps were unnecessary as far as getting the fat into solution was concerned (which is the object of these manipulations when dealing with blood), it was thought the results would be more conclusive if the whole process as applied to blood fat estimation were adhered to in estimating these known fat samples.

Thereafter the samples were subjected to exactly the same procedure as has been described for estimating blood fat.

It will be noticed that the amount of fat actually present in the flasks 1 and 1a at the outset was 6 mgrms.

After making the volume up to 50 cc. with alcohol-ether mixture only 25 cc. of this mixture was transferred to the conical flasks for analysis, i.e. the actual amount present for estimation was half the original value or 3 mgrms. Expressing this in terms of blood fat estimations where 3 mgrms represents the amount of fat in 1 cc. of blood we say the fat content of the sample is 300 mgrms. per 100 cc. or 300 mgrms. per cent. The procedure is thus absolutely parallel with the extraction of 2 cc. of blood and taking 25 cc. of the total 50 cc. volume thus taking up for estimation the amount of fat extracted from 1 cc. of blood.

The following tables give the results.

Table I.

Specimen	Known Fat Content mgms.per 100 cc.	Burette Reading	*Corrected Reading	Estimated Content mgms. per 100 cc.	Percentage Error
1	300	0.0135	0.0115	306.2	2.06
1	300	0.0132	0.0112	300.16	0.05
1a	300	0.0128	0.0108	289.44	3.52
1a	300	0.0130	0.011	294.8	1.73
2	600	0.024	0.022	589.6	1.73
2	600	0.025	0.023	616.4	2.73
2a	600	0.0237	0.0217	581.56	3.07
2a	600	0.0248	0.0228	611.04	1.84
3	900	0.0364	0.0344	921.92	2.43
3	900	0.0368	0.0348	932.64	3.62
3a	900	0.0362	0.0342	916.56	1.84
3a	900	0.037	0.035	938.0	4.22

Table I - Duplicate analyses of known concentrations of fat.

*The correction derived from macro- and micro-titration of the acid and alkali used, with a standard amount of titration fluid (1 c.c.) and a standard amount of indicator, was 0.002.

Table II.

Average error for each sample estimated (where two burette readings were taken)

Specimen	Known Fat Content. mgms.per 100 cc.	Burette Readings (Corrected)	Mean Burette Reading	Estimated Fat Content.	Percentage Error.
1	300	0.0115 0.0112	0.01135	304.18	1.39
1a	300	0.108 0.11	0.0109	292.12	2.62
2	600	0.022 0.023	0.0225	603.0	0.5
2a	600	0.0217 0.0228	0.02225	596.3	1.23
3	900	0.0344 0.0348	0.0346	927.28	3.03
3a	900	0.0342 0.035	0.0346	927.28	3.03
		Average Error for Duplicate Samples.			
1 and 1a	300	0.01135 0.0109	0.0111	297.48	0.84
2 and 2a	600	0.0225 0.02225	0.0223	597.64	0.39
3 and 3a	900	0.0346 0.0346	0.0346	927.28	3.03

Further, working with blood, duplicate samples were taken and put through the whole process independently.

The following results show how closely the estimations correspond despite the lengthy manipulations through which each sample has to go.

Table III.

Table III /

Table III.

Duplicate Samples (e.g. 1 and 1a - 2 and 2a) Put Through Whole Process Independently. Note Close Similarity of Results in Column II.

I.	II.	III.	IV.
Specimen	Estimated Fat Content. mgms. per 100 cc.	Average Estimated Fat Content.	Average Percentage Error.
1	435	439.5	1.1
1a	444		
2	592	597.5	0.9
2a	603		
3	600	598	0.3
3a	596		
4	698	698	0.0
4a	698		
5	1021	1024	0.3
5a	1027		
6	1027	1018	0.9
6a	1009		
7	275	273.5	0.6
7a	272		
8	577	577	0.0
8a	577		
9	479	476	0.7
9a	473		
10	565	560.5	0.9
10a	556		

These figures supply abundant evidence of the reliability of the method employed for fat estimation in this thesis. It must be emphasised again, however, how essential it is to have both reagents and apparatus accurately standardised.

Thus if the HCl be slightly below N/10 normal or if slightly less than 5.0 cc. be added the fatty acids will be incompletely liberated. Conversely, if acid be added in slight excess, it will remain to be neutralised in the titration. In either case apparently slight variations may lead to serious errors, notwithstanding the subsequent dilution, since the volume of the titrating fluid is so small. Careful manipulation is essential at every step but, granted this, the results are very satisfactory.

Despite the agreement of the duplicate samples, there still remains the possibility that the extract may contain, in addition to the fat and fatty acids, some acidic substances soluble in aqueous alcohol-ether mixtures as well as in water. In this category would be included such substances as acetoacetic acid, hydroxybutyric acid and so on. These, though present only in traces in normal blood may occur to an appreciable extent in pathological specimens. They are, however, of low boiling point and are volatile in steam so that they are removed by the boiling down after the addition/

addition of hydrochloric acid. An experiment performed by Stewart and White (1925) showed that interfering substances were absent and the part they play in the estimation of total fatty acid absolutely negligible.

RESULTS

Section I :

The Fasting Level of the Blood Fat in Abnormal Conditions.

RESULTS - Section I :

The Fasting Level of the Blood Fat in Abnormal Conditions.

It was decided to investigate whether or not any marked disparity existed between the fasting levels of the blood fat in various pathological conditions when due attention was paid to the effect of exercise on the blood fat content and when such effect was eliminated by adequate rest.

Consequently each patient in the following section, whether in-patient or out-patient, was subjected to an hour's complete rest (lying down on a couch) before the sample was withdrawn.

Each patient, moreover, was instructed to take a light meal at 8 p.m. on the evening before the test and to take nothing more after this time. Thus a full 12 hours' fast was assured in each subject.

It was decided to investigate (1) a series of "normals" - first and second year students who volunteered their services; (2) a series of "over-weight people, who, it was felt, would possibly show an increased blood fat content, and (3) a series of "nervous disease" patients where the nervous affection manifested itself in definite tremor. These people were selected since it was felt that many of these cases showed a disappearance of the myelin sheath/

sheath and that such disappearance (according to some observers) resulted in lack of insulation of the conducting nerve and consequent tremor. It was thought that the disappearance of the myelin sheath might be associated with a low blood fat content.

Table IV shows the results of estimation. Left hand section shows the fasting levels of the obese cases

Middle section " " " normal cases

Right hand section " " " nervous cases

Corresponding with the patient's case number and initials will be found a few clinical notes on the case under the headings "Obesity, "Normals" and "Nervous".

Table IV.

Obesity			Normals			Nervous		
Case no.	Initials	Fat mg. per 100 cc.	Case no.	Initials	Fat mg. per 100 cc.	Case no.	Initials	Fat mg. per 100 cc.
1	M.F.	2042.4 +	1	M.C.M.	439.5	1	W.A.	301.9
2	J.M.	1693.1 +	2.	E.M.F.	661.5	2	A.R.	331.5
3	I.H.	1574.7	3	R.W.	698.5	3	R.P.	449.9
4	M.E.	1263.9 +	4	J.C.B.	560.9	4	A.L.	242.7
5	G.I.	2033.5	5	P.N.C.	476.5	5	G.Y.	275.2
6	E.N.	1302.4	6	H.T.C.	695.6	6	F.S.	198.3
7	J.W.	1825.0	7	A.J.	695.6	7	W.G.	366.3
8	E.C.	2619.6	8	W.M.A.	612.7	8	R.H.	180.5
9	N.V.	1593.0	9	I.H.M.	535.7	9	J.H.	410.7
10	F.	1500.0	10	L.W.F.	645.8	10	D.D.	305.0
Average		1744.76	Average		602.2	Average		306.2

Showing a comparison between the blood fat fasting levels of obese subjects, normal subjects and nervous disease subjects. Ten examples are given in each class.

Table V.

Case No.	Initial	Fat. Mg. per 100 cc.
1	W.B.	2164.5
2	P.A.	2153.4
3	P.W.	1909.2

Showing the high blood fat fasting levels recorded in nephritic subjects.

Short Clinical Notes on Subjects Examined.

Table IV. The "normals" in Table IV were all healthy first and second-year medical students actively engaging in athletics and perfectly free from any obvious abnormality.

The "nervous" cases were:

Case 1. W.A. (5.4.27) Ward 31, R.I.E. Age 54. Diagnosis disseminated sclerosis. Intention tremor of hands; slight ataxia of feet. On full ward diet. Weight 13 stones and "getting stout". No kidney symptoms.

Case 2. A.R. (5.4.27) Ward 31, R.I.E. Age 44. Suffering from "Ataxia with diabetes". Coarse tremor of arms of 14 months' duration. Acetone and sugar present in urine. No symptoms of diabetes. Weight 10st.9 lbs. Diet 1000 calories G:F.A, ratio = 1.1. Diagnosis: The glycosuria and ataxia were caused by the same condition, the lesion probably being situated in some part of the brain stem.

Case 3. R.P. (7.4.27) Ward 31, R.I.E. Age 19. Diagnosis- post encephalitis lethargica; marked rigidity of limbs: muscles of expression sluggish. Weight 8st.13lbs. No kidney symptoms. Ordinary full ward diet.

Case 4. A.L. (5.4.27) Ward 31, R.I.E. Age 34. Diagnosis - "War Neurosis". Constant facial movements and involuntary spasmodic movements of hands, arms and head. Weight 7st.9lbs. Full ward diet.

Case 5. G.Y. (7.4.27) Ward 31, R.I.E. Age 37. Diagnosis- Post Encephalitis Lethargica. Tremor of hands and arms. Weight 8st.12¼lbs. Full ward diet. No kidney symptoms.

Case 6. F.S. (21.4.27) Ward 31, R.I.E. Age 42. Diagnosis - Disseminated Sclerosis, ataxia of both legs on purposive movement. Intention tremor not definite. Weight 11st. 3 lbs. Full ward diet. No kidney symptoms.

Case/

Case 7. W.G. (18.11.25) Ward 28, R.I.E. Age 45. Diagnosis- Disseminated Sclerosis. Marked intention tremor. Marked wasting of lower extremities and marked ataxia. Patient bed-ridden. No kidney symptoms. On full ward diet.

Case 8. R.H. (18.11.25) Ward 28, R.I.E. Age 34. Diagnosis- Early G.P.I. Presented symptoms of spastic hemoplegia passing off in two days . Wasserman ve and history of infection. Weight 10st.¼ lbs. On full ward diet. No kidney symptoms.

Case 9. J.H. (20.11.25) Ward 28, R.I.E. Age 17. Diagnosis - "Chorea". Uncontrollable and purposeless twitchings of left arm, left leg and left side of face. Weight 8 st. 1½ lbs. Nutrition good. On full ward diet. No kidney symptoms.

Case 10.D.D. (20.11.25) Ward 28, R.I.E. Age 19. Diagnosis Post Encephalitis Lethargica. General rigidity of muscles; typical mask-like expression. General wasting of all muscles and disappearance of subcutaneous fat. On full ward diet. Weight 7 st. 2¼ lbs. No kidney symptoms.

The "Obesity" cases were:-

Case 1. M.F. Date 22.6.27. Investigated in ward 25 in Feb. 1923 for pituitary involvement. X-ray at this time showed sella of much enclosed type with flattened floor. Posterior clinoid processes thin looking and somewhat curled over. No urinary sugar was present at this time but appeared later and patient is at the time of examination attending the diabetic clinique of the R.I.E. She has been sugar free for some months and is on a low caloric diet (1500).

Fat distribution is mainly on thighs, abdomen and lower part of the chest. The arms and legs are not particularly affected.
Age 40, weight 14 st. 8 lbs.

Case/

Case 2. J.M. Date 22.6.27. Attending the diabetic clinique of the R.I.E. for past three years. Has had no sugar or acetone for nearly one year. Has not had insulin. On low caloric diet (810) made up of 60 grams protein, 55 grams carbohydrate and 30 grams of fat. Fat distribution general. At present taking 2 gr. Thyroid t.i.d.
Age 45, weight 15st. 12 lbs.

Case 3. I.H. Patient reporting monthly after having been in Ward 25, R.I.E. Diagnosis was "Obesity due to polyglandular deficiency". Fat distribution general. An enormous woman unable to walk on account of her weight. Taking Ext. Thyroid gr. v t.i.d. for 7 days, then leaving it for 7 days. At time of examination was not taking thyroid. Diet 1500 calories (with protein 80 grams).
Age 35, weight 22st. 7 lbs.

Case 4. M.E. Date 27.6.27. Patient has been undergoing treatment as a diabetic for 4 years and has constantly shown sugar despite treatment. She is a hopeless case (apparently) for no arguments will persuade her to stick to her diet. She puts on weight on a 500 caloric diet. At time of examination is supposed to be on a 700 calorie diet.
Age 50, weight 12 st.

Case 5. G.T. Date 29.6.27. An untreated case of pure obesity. Patient came up with her sister and gave a blood sample as a favour. Complaining of nothing save being "too fat". Healthy appetite great meat and potato eater. Sample taken was, of course, a fasting sample.
Age 24, weight 14 st.

Case 6. E.N. Date 27.6.27. Patient treated in Ward 25 in 1920. At time of examination was undergoing no treatment beyond keeping on low diet of about 1500 calories. Had been treated for obesity in 1920 and came up for a blood/

(Case 6 contd.) blood test in response to a request from us. Feeling quite fit but still overweight. No special distribution of fat.
Age 42, weight 15st. 2 lbs.

Case 7. J.W. Date 10.12.25. Patient in Ward 33, R.I.E. undergoing investigation from point of view of pituitary lesion. Distribution of fat not suggestive of pituitary type but rather a generalised adiposity. Patient on low fat diet.
Age 16, weight 17 st.

Case 8. E.C. Date 16.10.27. In-patient Ward 27, R.I.E. Diagnosis was that the case was initially one of exophthalmic goitre followed later by deficiency of thyroid secretion producing the present picture of myxoedema. Glycosuria has lately developed. B.M.R. at this time was -18%.
Age 48, weight 14st. 8 lbs.

Case 9. N.V. Date 7.11.27. In-patient Ward 27, R.I.E. A ward maid in the Infirmary showing a definite myxoedema. Weight 13 st. 8 lbs.

Case 10. Mrs.F. 15.11.27. In-patient in Ward 27, R.I.E. Case of myxoedema. No particular distribution of fat - fairly general. Weight 13st. 4 lbs.

Clinical /

Clinical Data - Table V.

Case 1. W.B. Patient admitted 26.11.26 in unconscious state. Diagnosed as uraemia. Died without recovering consciousness three days later. Patient muscular and stout. Age 43.

Case 2. P.A. 27.11.26. Boy aged 15 in Ward 26, R.I.E. suffering from nephritis. Blood withdrawn showed definite lipaemia on standing for a minute or two. This patient died shortly afterwards and p.m. was found to have kidneys of infantile development.

Case 3. P.W. 27.11.26. Patient in Ward 26, R.I.E. Generalised arterio sclerosis of advanced type. Albuminuria. Weight 16 st. 7 lbs. Age 63.

Discussion.

While this part of the investigation is by no means complete or conclusive, the marked disparity between the above averages for each section is, at least, arresting.

It should be noted that, apart from the averages being widely divergent, each of the whole series of "nervous" cases, for example, is low when compared with the "normal" while each of the "obesity" cases is high.

Although such variation may occur from day to day in individual cases in pathological conditions (a nervous case examined to-day may be low and to-morrow high), it seems hardly probable that the samples were withdrawn in the whole series of nervous cases when at their lowest and from the whole series of obese cases when at their highest fat content.

(N.B. It will be shown that the individual daily variation of the fasting fat content in normals is remarkably constant. See Section II).

Consequently one may justifiably assume that the fasting blood fat content is high in obesity and low in nervous disease associated with tremor. This is particularly important in view of the association of obesity with diabetes mellitus - that stage of obesity recognised clinically by many as the "pre-diabetic obesity."

The/

The low readings found in nervous cases would seem to be an indication in support of the pushing of fat in the diet - the basis of the Weir-Mitchell treatment of over feeding.

(It should further be noted that in the selection of the above cases the presence of additional complications was avoided as far as possible. In particular the absence of any kidney condition was insisted upon since it was in connection with nephritis that some of the highest recorded fat fasting levels were noted (see Table V).

RESULTS.

Section II - Blood Fat and Exercise in Health.

II. Blood Fat and Exercise in Health.

Introduction.

It has been explained already how the effect of exercise was noted accidentally in the course of auto-experimentation on the part of the author. It was felt that this factor should be verified in other cases before entering into a more detailed investigation. Table VI shows the results of experiments on four volunteer medical students who came up to the laboratory without breakfast, having subjected themselves to a previous 12 hours' fast.

Specimen A is the fasting sample first withdrawn and after its withdrawal the patient ran round the hospital for 15 minutes. Immediately after the exercise specimen B was collected. The results amply confirmed the indication of a rise in blood fat after exercise and it was determined to proceed with a more thorough investigation of the subject.

Since the subjects (medical students)lived at varying distances from the laboratory, it was decided to eliminate any error from increase of blood fat consequent upon the exercise of reaching the laboratory by subjecting the patient to an hour's complete rest before withdrawing any blood sample.

Blood sugar and cholesterol variations were also/

also investigated in this series of cases and the respiratory quotient and blood concentration estimated in two or three cases.

The blood sugar was estimated by Benedict's method (1918). The results are all high and in later investigations the method of Folin and Wu (1920) has been used. In a series of estimations comparing the results of estimation by both these methods, the Benedict technique has been observed to give results consistently 20% above those by the Folin-Wu method.

The cholesterol content was determined by Bloor's modification (1915) of the Autenrieth-Funk process(1913).

The air samples for the determination of the respiratory quotient were collected by means of the Douglas Bag (1911) with subsequent analysis by means of Haldane's (1918) gas analysis apparatus.

The percentage of the corpuscles was estimated by the haematocrit.

Results.

Preliminary experiments verifying the occurrence of a rise in blood fat content after exercise are detailed in Table VI.

Table VI /

Table VI.

Patient	Athletic type.	Date	Specimen	Remarks	Time	Fat.mgms. per 100 cc.	%age increase.
L.W. Foster	1st XI Hockey	30/10/26	A	Fasting	9 a.m.	645.8	
			B	After exercise	9.15 a.m.	1015.2	57
D. Fraser	do.	30/10/26	A	Fasting	9.5 a.m.	932.4	
			B	After exercise	9.20a.m.	1243.2	23
R. Walmsley	Keen walker	15/11/26	A	Fasting	9.30a.m.	698.5	
			B	After exercise	9.45a.m.	1184.0	69
H.T. Campbell	Rugby	26/11/26	A	Fasting	9.45a.m.	695.6	
			B	After exercise	10 a.m.	1260.9	81

Table VI - Showing increase in fasting blood fat content after exercise in normals. The exercise consisted of $\frac{1}{4}$ hour's running round the hospital.

Undue stress could not be laid upon this variation of blood fat after exercise unless some constant of blood fat content be found, for the increase might be due to normal fluctuation of the blood fat level. Experiments (Table VII) showed that the fasting fat content remained remarkably constant for a given individual over a considerable period of time.

Table VII /

Table VII.

Subject	Date	Fasting level mg. per 100 cc.	% variation
E.M.F.	Nov. 17, 1926	661	
	Feb. 16, 1927	615	6.5
I.H.M.	Dec. 4 , 1926	535	3.0
	Feb. 28, 1927	520	
A.J.	Nov. 27, 1926	696	1.0
	Mar. 2 , 1927	690	
W.M.A.	Dec. 3, 1926	612	3.0
	Feb. 14, 1927	627	
M.C.M.	Nov. 12, 1926	439	1.5
	Feb. 10, 1927	433	

Having established a constant it was now possible to go forward with a more thorough investigation of exercise effect. Table VIII shows the results of the effect of exercise in these cases.

In each individual case the same amount of exercise has been performed (1) in the fasting state, (2) after the administration of 100 g. glucose by the mouth. In all cases specimen A is a basal fasting sample. In the experiments where glucose has been given the 100 g. are taken immediately after the collection of specimen A, and specimens B and C are collected at intervals of $\frac{1}{2}$ and 1 hour respectively, the patient resting in the interval. Specimen D is in all cases the sample immediately after exercise. Specimens E and F are collected at the times noted with/

with the patient resting after exercise.

The subjects of the experiments were all healthy medical students.

Figs. V to X represent in graphic form the experimental data of Table VIII. The number of the figure is noted in the right hand column opposite the corresponding case.

Table VIII /

Table VIII.

Case	Specimen	Remarks	Time	Fat (mg. per 100 cc.)	Sugar (mg. per 100 cc.)	Cholesterol (mg. per 100 cc.)	% Corpuscles in blood.	R.Q.	Work Done	Graph
(1)* E.M.F.	A	Fasting	9.35 a.m.	661.5	-	169.4	-	0.739	Running along definite route for 15 mins.	Fig. V
	D	After exercise	9.55	947.2	-	202.0	-	0.775		
	E	Resting	10.15	779.9	-	173.9	-	0.776		
	F	Resting	11.45	609.7	-	212.7	-	0.726		
(2)†	A	Fasting	10.10	615.6	119.0	-	38		Running along same route for same time.	Fig. VI
	B	½ hr.after sugar	10.40	627.5	154.0	-	-			
	C	1 hr. do.	11.10	577.2	129.0	-	32			
	D	After exercise	11.30	571.2	285.0	-	52			
	E	Resting	12.5 p.m.	461.7	222.0	-	-			
(1)* I.H.M.	A	Fasting	10.30a.m.	520.9	160.0	-	34		Bicycle ergometer. Work done = 28,632 kg.metres.	
	D	After exercise	11.0	701.5	270.0	-	38			
	E	Resting	11.5	734.0	116.0	-	57			
	F	Resting	11.30	686.7	163.0	-	46			
(2)†	A	Fasting	10.15a.m.	535.7	-	200.0	-		Bicycle ergometer. Repetition of above exercise. Work done = 28,632 kg.metres.	
	B	½ hr.after sugar	-	-	-	-				
	C	1 hr. do.	11.15	515.0	-	215.0				
	D	After exercise	11.40	467.6	-	217.0				
	E	Resting	12.20p.m.	518.0	-	208.0				
(1)* A.J.	A	Fasting	10.45a.m.	695.6	138.0	-	40		Bicycle ergometer. Work done = 37,794 kg.metres.	
	D	After exercise	11.0	843.6	235.0	-	39.6			
	E	Resting	11.25	858,4	253.0	-	40			
	F	Resting	11.35	666.0	202.0	-	38			
(2)†	A	Fasting	10.10	689.6	126.0	208.3	-		Bicycle ergometer. Work done = 37,794 kg. metres. (Repetition of above exercise)	
	B	½ hr.after sugar	-	-	-	-	-			
	C	1 hr. do.	11.10	680.8	235.0	204.0	-			
	D	After exercise	11.25	710.4	135.0	204.0	-			
	E	Resting	12.15p.m.	666.0	154.0	212.7	-			
(1)* W.M.A.	A	Fasting	10.18a.m.	627.5	160.0	-			Bicycle ergometer. Work done = 57,266 kg.metres.	Fig. VII
	D	After exercise	10.40	864.3	227.0	-	-			
	E	Resting	11.10	488.4	175.4	-	-			
	F	Resting	11.30	370.0	196.0	-	-			
(2)†	A	Fasting	9.50	612.7	-	1980	-		Bicycle ergometer. Repetition of above exercise. Work done = 57,266 kg. metres.	Fig. VIII.
	B	½ hr.after sugar	-	-	-	-				
	C	1 hr. do.	10.50	603.8	-	190.4				
	D	After exercise	11.20	580.6	-	202.0				
	E	Resting	-	-	-	-				

(1)/

* Without Glucose † With Glucose

102.

Table VIII (contd.)

Case	Specimen.	Remarks	Time	Fat (mg. per 100 cc.)	Sugar (mg. per 100 cc.)	Cholesterol (mg. per 100 cc.)	% Corpuscles in blood.	R.Q.	Work Done	Graph
(1)* M.C.M.	A	Fasting	9.20a.m.	439.5	-	210.4	-	0.763	Running along definite route for 15 mins.	Fig. IX
	D	After exercise	9.35	597.9	-	197.2	-	0.798		
	E	Resting	10.5	630.4	-	206.9	-	0.787		
	F	Resting	10.40	598.8	-	200.0	-	-		
(2)†	A	Fasting	10.13	433.6	120.0	-	53		Running along same route for same time.	Fig. X.
	B	½ hr. after sugar	10.43	245.6	206.2	-	46			
	C	1 hr. do.	11.13	553.5	176.9	-	54			
	D	After exercise	11.32	429.2	281.7	-	-			
	E	Resting	11.57	432.1	217.3	-	42			

*Without glucose †With glucose

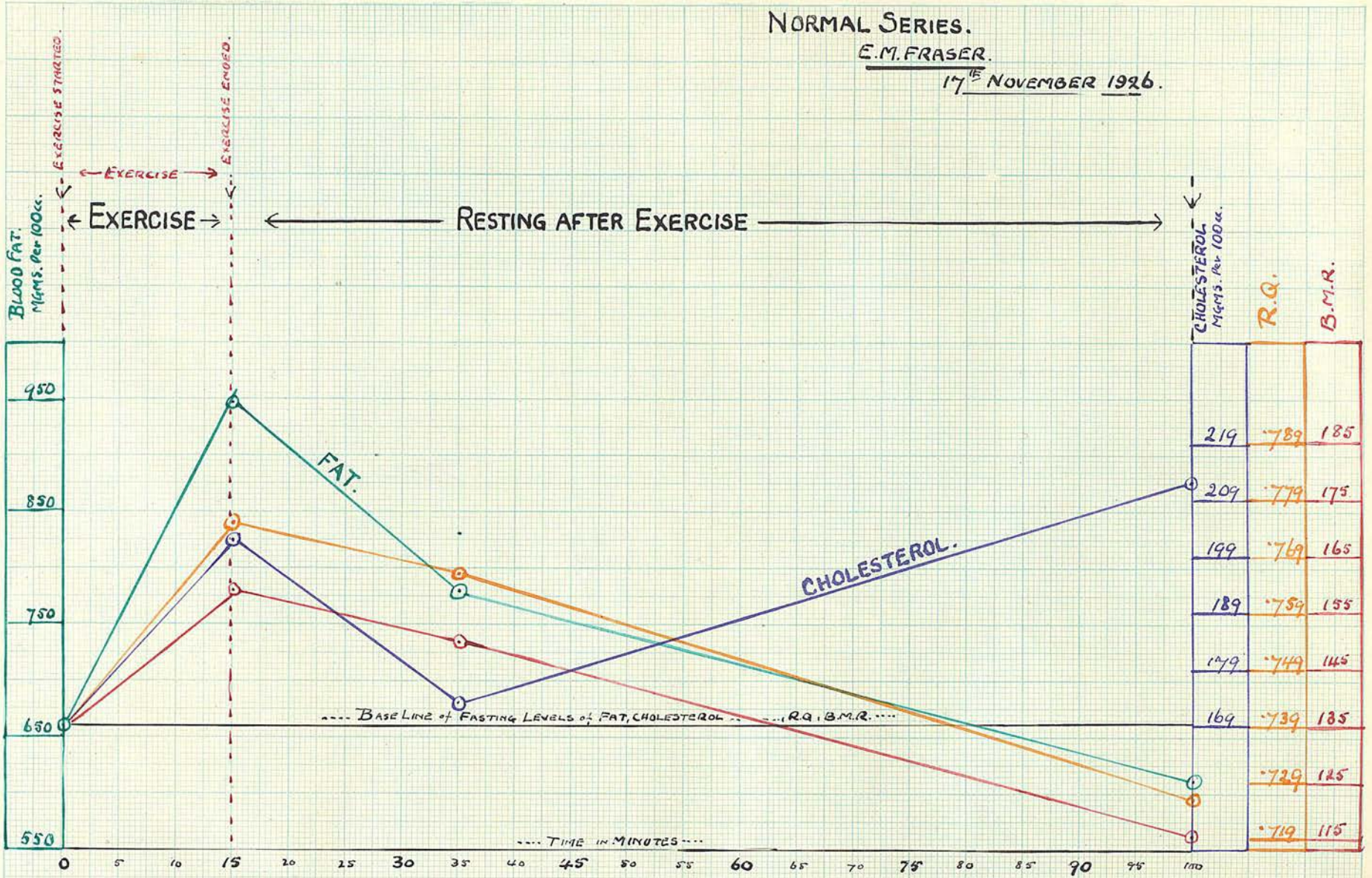

NORMAL SERIES.
E.M. FRASER.
17TH NOVEMBER 1926.
EXERCISE STARTED.
EXERCISE ENDED.
← EXERCISE →
← EXERCISE →
← RESTING AFTER EXERCISE →
BLOOD FAT. MGMS. PER 100cc.
CHOLESTEROL MGMS. PER 100cc.
R.Q.
B.M.R.
950
850
750
650
550
FAT.
CHOLESTEROL.
---- BASE LINE of FASTING LEVELS of FAT, CHOLESTEROL ---- ...R.Q. : B.M.R. ....
.... TIME IN MINUTES ....
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
219 .789 185
209 .779 175
199 .769 165
189 .759 155
179 .749 145
169 .739 135
.729 125
.719 115

FIGURE V

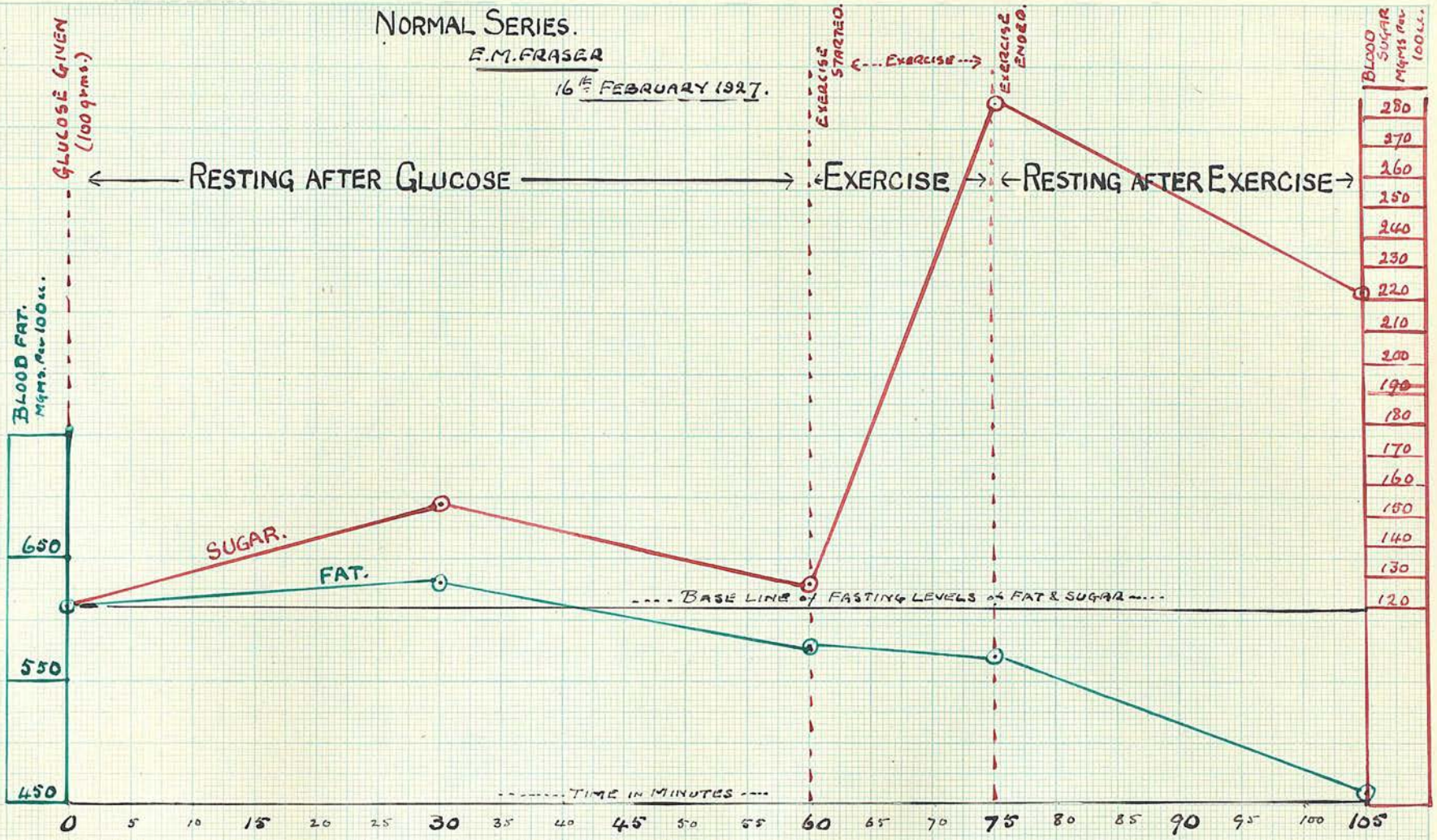

NORMAL SERIES.
E.M.FRASER
16th FEBRUARY 1927.
GLUCOSE GIVEN (100 grms.)
EXERCISE STARTED.
EXERCISE ENDED.
←--- EXERCISE --->
BLOOD SUGAR Mgms per 100 cc.
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
← RESTING AFTER GLUCOSE →
← EXERCISE →
← RESTING AFTER EXERCISE →
BLOOD FAT. Mgms. per 100 cc.
650
550
450
SUGAR.
FAT.
---- BASE LINE of FASTING LEVELS of FAT & SUGAR ----
---------- TIME IN MINUTES ----
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105

FIGURE VI

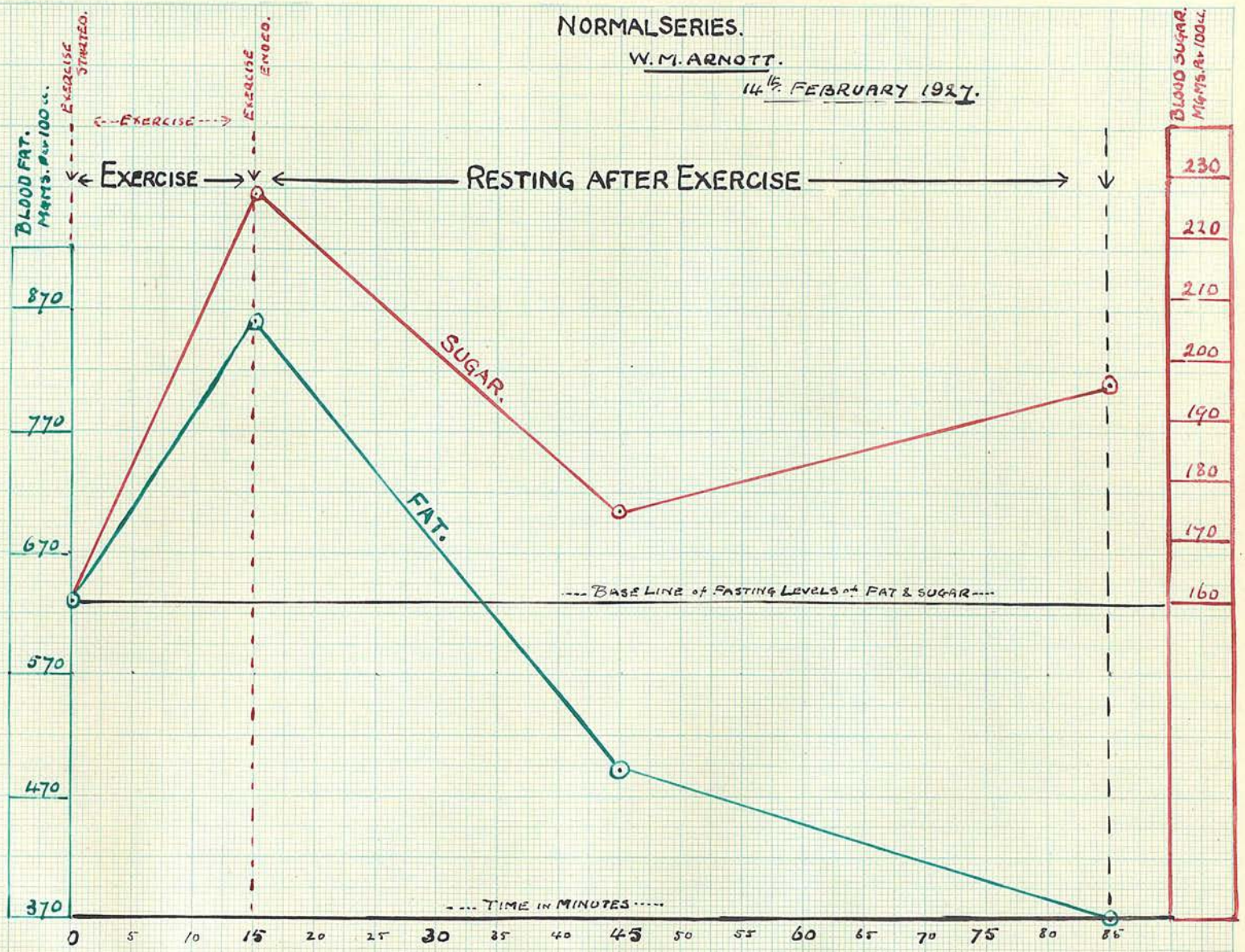

NORMAL SERIES.
W. M. ARNOTT.
14th FEBRUARY 1927.
BLOOD FAT. Mgms. per 100 cc.
BLOOD SUGAR. Mgms. per 100 cc.
EXERCISE STARTED.
EXERCISE ENDED.
EXERCISE
RESTING AFTER EXERCISE
SUGAR.
FAT.
BASE LINE of FASTING LEVELS of FAT & SUGAR
TIME IN MINUTES
870
770
670
570
470
370
230
220
210
200
190
180
170
160
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85

FIGURE VII

NORMAL SERIES.
CASE W.M.A.
3·12·26.

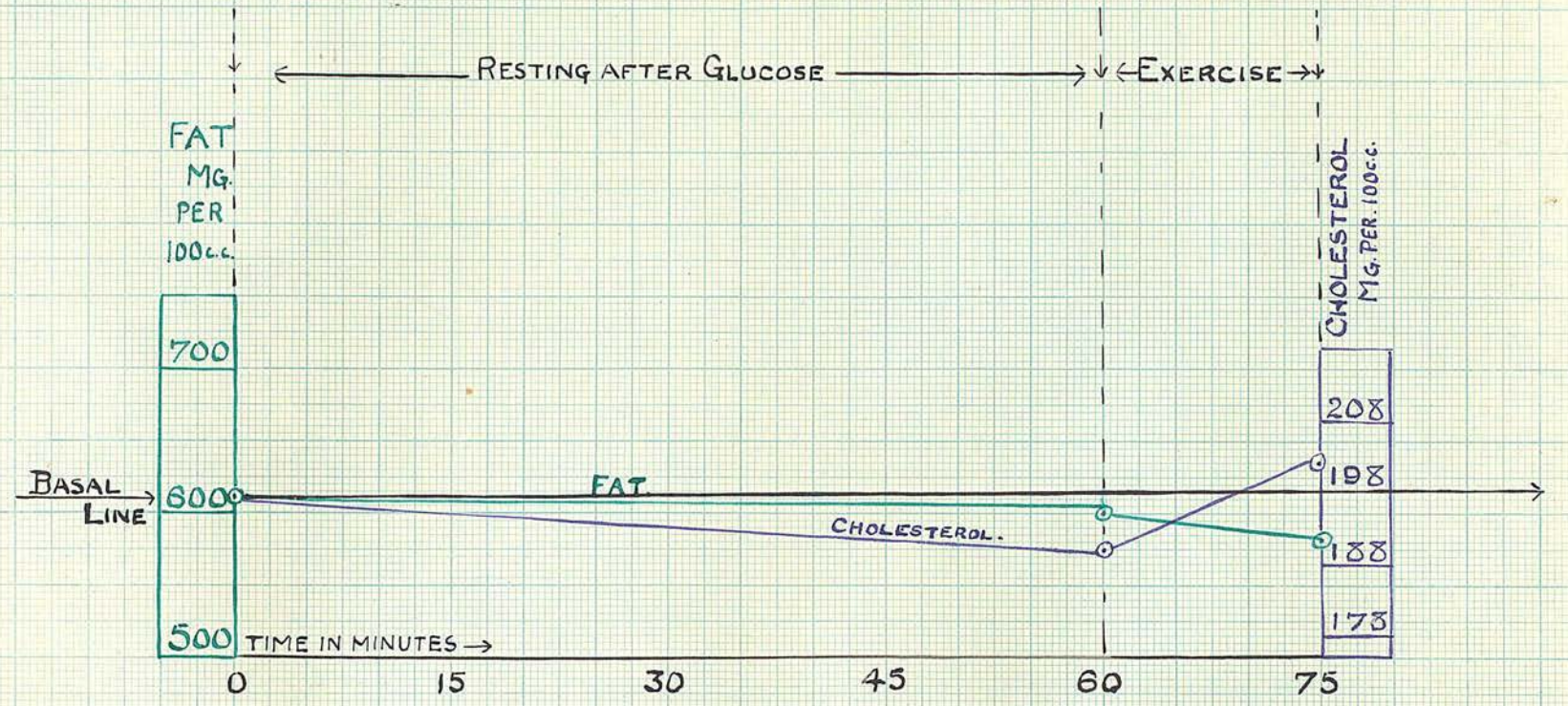


FIGURE VIII

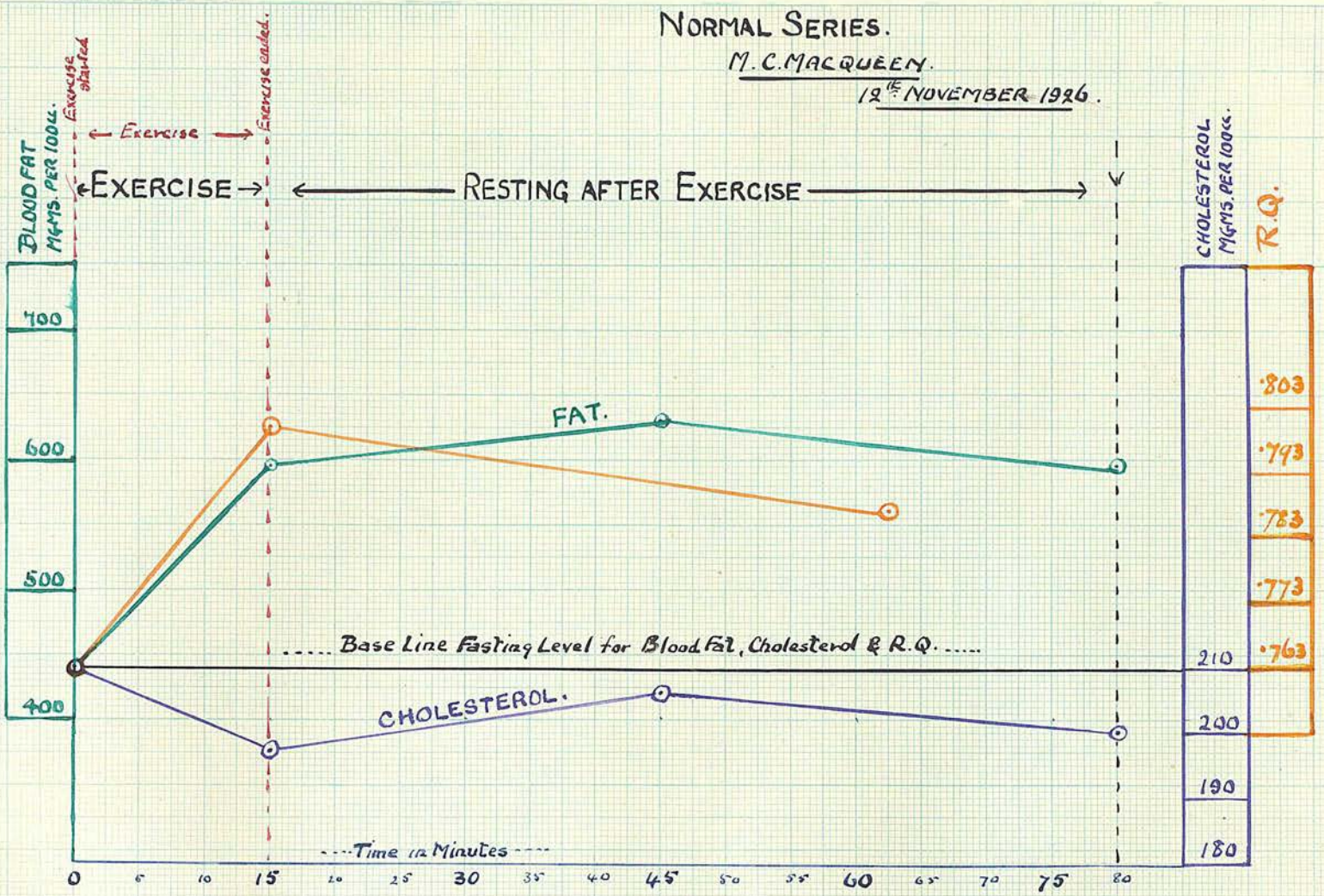

NORMAL SERIES.
M. C. MACQUEEN.
12th NOVEMBER 1926.
BLOOD FAT MGMS. PER 100 cc.
Exercise started.
Exercise ended.
← Exercise →
←EXERCISE→
←——— RESTING AFTER EXERCISE ———→
CHOLESTEROL MGMS. PER 100 cc.
R.Q.
700
600
500
400
FAT.
.803
.793
.783
.773
.763
..... Base Line Fasting Level for Blood Fat, Cholesterol & R.Q. .....
210
CHOLESTEROL.
200
190
180
---Time in Minutes---
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80

FIGURE X

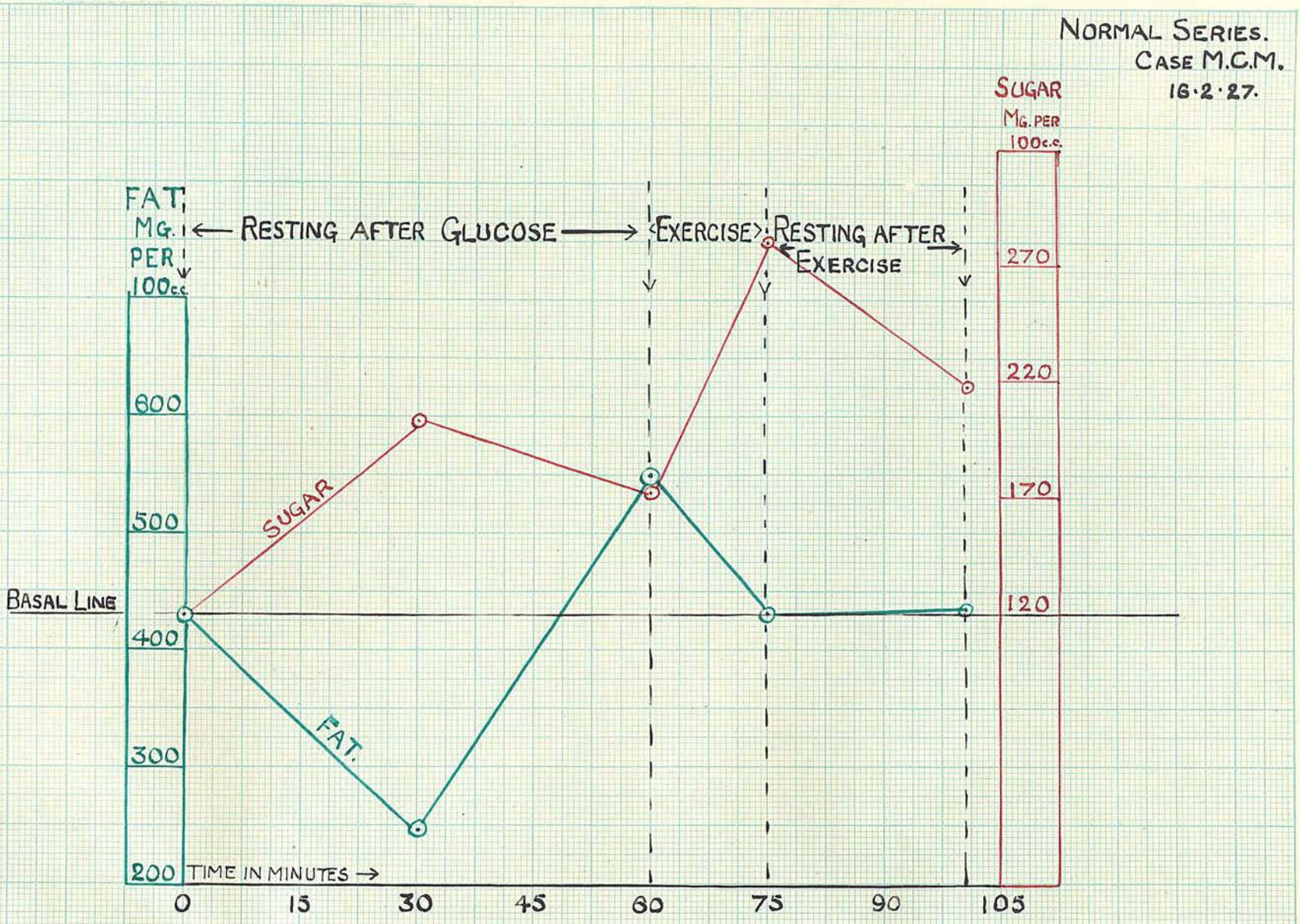

NORMAL SERIES.
CASE M.C.M.
16·2·27.
SUGAR
MG. PER
100c.c.
FAT
MG.
PER
100c.c.
RESTING AFTER GLUCOSE
EXERCISE
RESTING AFTER EXERCISE
270
220
170
120
600
500
400
300
200
BASAL LINE
SUGAR
FAT.
TIME IN MINUTES →
0
15
30
45
60
75
90
105

FIGURE X

Discussion.

The most striking feature of these experiments is the constant increase in the blood-fat content after exercise in the fasting individual (Figs. V, VII and IX), an increase which does not occur after the ingestion of glucose (Figs. VI, VIII and X).

The increase obtained is well beyond the margin of experimental error. Thus in case E.M.F. the blood fat content is increased by 43%, in I.H.M. by 35%, in A.J. by 21%, in W.M.A. by 37%, and in M.C.M. by 36%.

It may be argued that this increase is due entirely to the concentration of the blood consequent upon the severe exercise, but in cases E.M.F. and M.C.M., where this question was investigated, the increase in concentration of the blood corpuscles was found to be 4% in the former and nil in the latter, while the increase in fat content amounted to 35% and 21% respectively. Similar findings have resulted from the investigation of this point in cases not yet published, so that one is in a position to state that the registered increase of blood fat after exercise is greater than that which would result from simple concentration of the blood after exercise and is, in fact, a real and not an apparent increase.

Having established the fact of a definite

increase in blood fat after exercise, one or two other points require to be settled before discussing its significance.

In the first place Leathes and Raper (1925) do not regard the variations of blood fat content in samples of blood taken "from any accessible vein" as being of any value whatsoever in determining the nature and mechanism of fat transport and utilisation. The significance of such data derived from examination of the blood is discussed in a paper by Lyon (1923) dealing with some factors which influence dosage. Secondly, before a variation in blood fat content can be of any importance a constant must be established. If we have no constant the increase in blood fat after exercise loses much of its significance, since such increase might be due to normal fluctuation of the blood fat level.

The Constancy of the Blood-Fat Content in Fasting Individuals.

Most observers hitherto have found extreme variations in the blood fat fasting content in successive examinations and White (1926) has mentioned the possible factors influencing the blood fat level and "the difficulty of obtaining any definite correlation between them."

Terroine, on the other hand (1914), working with dogs, found that any one dog would show a similar amount of fat in the blood provided the conditions/

ditions remained similar (the amount being subject to a variation of 15% either way). He found considerable variation, however, in comparing the fat contents of different dogs under similar conditions. In other words, subject to a variation of 15%, the individual fasting fat content remained steady under similar conditions. This latter view is borne out by the results of this series of experiments on men, save that here the percentage variation is much less. Thus it will be noted (Table VII) that while the range of fasting blood fat content in the five cases of normal students is from 439 mg. to 695 mg. per 100 cc., the fasting blood fat level in the individual shows a definite tendency to remain constant, and that the increase of blood fat after exercise is a real and not an apparent increase.

From Table VIII it is evident that a very definite increase in the blood fat content occurs after exercise in the fasting individual. It is realised that the exercise involved in a patient moving from one room to another is sufficient to upset the estimations of basal metabolism and blood pressure for an hour afterwards. It would appear, then, that the variations in fasting blood fat content found hitherto by other observers may be accounted for by the fact that insufficient attention had been paid to the importance of the effect of exercise as a disturbing factor.

No basal sample was withdrawn in any of the present series of cases until the patient had rested on a couch for an hour.

The increase of the blood fat content after exercise.

This increase may be due to either of two factors. It may be a definite response to a demand for more fat on the part of the working cells, or it may be due to a non-removal of the fat from the blood stream, the working cells either refusing it or being unable to utilise it.

The first hypothesis appears to be the more logical.

This increase in blood fat content after exercise has been remarked upon by Gage and Fish (1924), who determined the number of fat particles in the blood by microscopic dark-field, high-power investigation. While the method seems somewhat crude, these observers noted a definite increase in fat particles, or "chylomicrons" as they call them, after exercise, and they interpreted the results as meaning that in the ordinary activity of a fasting man the fat is poured out into the blood from the depôts only as it is needed and at the rate it is needed.

They found that it took some time for the fat content to resume normal proportions, and liken the effect of exercise to that of absorption after a fatty/

fatty meal where the return of the fat to its previous level is slow, the fall being due to "metabolism or assimilation or both as in the curve of digestion."

While this point of the slow return of the blood fat content to fasting level was not investigated in this series of experiments, the indications are not in support of this finding, for in no case was the examination maintained beyond 2 hours after the exercise, and yet in three of the five cases the blood fat content had already returned to, or gone lower than, its previous fasting level.

The Variations of the Blood Fat Content after Exercise when 100 g. Glucose had been taken prior to the Exertion.

The increase in fat content does not take place if the patient has had glucose before the exercise, despite the fact that the amount and the severity of the exercise were the same in both the investigations with glucose and without it. In all cases the fat levels after exercise when glucose has been previously taken remain practically constant (Figs. VI, VIII and X).

This suggests that the working cells only call upon the fat when they have insufficient carbohydrate readily available.

The/

The Respiratory Quotient in Relation to Exercise.

The existing views on this question are somewhat conflicting and not only do the interpretations of experimental data differ, but the actual results of similar experiments vary greatly.

Some observers (see Chauveau, 1896) maintain that carbohydrate alone furnishes the energy of muscular work and that fat can only be utilised by first undergoing conversion into carbohydrate.

Others (Zuntz, 1896; Loewy, 1908; Katzenstein, 1891) find no difference in R.Q. before and during muscular work, or (Benedict and Cathcart, 1914) maintain that this is true only for light work, and that during heavy muscular work the R.Q. is slightly raised.

Another theory (Atwater and Benedict, 1903) is that the working cells utilise fat and carbohydrate in the proportions in which they are presented to them, and that if fat is to be used in the production of muscular energy, then it is oxidised directly and does not undergo a preliminary conversion into sugar.

More recently Krogh and Lindhard (1920, 1, 2) suggest that during rest and during work the proportion of fat and carbohydrate catabolised is a function of the available supplies of these substances, and that carbohydrate is formed from fat and stored when the R.Q. is below 0.8, while a corresponding/

corresponding transformation of carbohydrate to fat takes place when the R.Q. is above 0.9.

Benedict and Cathcart (1914) find that the R.Q. rises during prolonged and heavy work, but when the work is finished the R.Q. falls below that observed previous to the performance of the work.

The investigations into this question in the present series of cases are not sufficiently extensive to warrant any sweeping deductions. In the two cases in which the R.Q. was followed up (cases E.M.F. and M.C.M.) it will be noticed (a) that the initial basal R.Q. is low, (b) that immediately after exercise the quotient tends to rise slightly, and (c) after an interval of rest the R.Q. has fallen again (in one case (Fig. V) it has fallen below that observed previous to the performance of the exercise).

Taking into account the noted increase in blood fat after exercise, one might interpret these changes as follows.

(i) That, on the basis of the observations of Krogh and Lindhard, in the initial fasting period the low R.Q. suggests that fat is being converted into carbohydrate and stored.

(ii) That during exercise the stored carbohydrate is utilised, thus tending to force the quotient up towards unity, which is the R.Q. for pure carbohydrate combustion. Coincidently, more fat/

fat is poured out for conversion into sugar to maintain the supply of carbohydrate, so tending to lower the R.Q. The resultant slight increase in R.Q. noted immediately after exercise is the mean of these two effects.

(Note. The R.Q. was not taken during exercise, but immediately after it, when, it may be assumed, the physiological mechanism at work during exercise had not had time to readjust itself to resting conditions).

(iii) After exercise, during the resting period, the depleted carbohydrate store is restored by the transformation of fat into carbohydrate with the consequent fall in R.Q. which such interconversion entails.

The Relationship between the Blood Sugar and Blood Fat Contents.

Although our knowledge at present remains insufficient for any very definite conclusions to be reached, there are certain indications that the blood sugar and blood fat contents are in some way related.

The evidence for such a relationship adduced in the present investigations may be briefly summarised as follows.

(1) In case M.C.M. it is noticed that after the taking of glucose (the individual resting), while the blood sugar rises in the first half-hour and falls in the next half-hour, the blood fat content/

content does exactly the reverse. This has proved to be a fairly constant factor in many cases not yet published (Fig. II).

A somewhat similar inter-relationship between blood fat and blood sugar has been noted by Oliver and Haworth (1923) who found a fall in blood fat occurring after the administration of glucose. The fall was more prolonged than in the present case.

(2) This apparent relationship in the resting individual between the blood sugar and blood fat, where, during absorption of sugar, the peak of the blood sugar surve coincides with the trough of the blood fat curve, is not maintained during exercise.

Here all the cases of exercise without glucose show an increase of blood sugar side by side with an increase of blood fat immediately after exercise, while in those cases where glucose was given before the exercise was started we have no increase in blood fat but definite rise of blood sugar in two cases and a definite fall in another case. These changes are quite definite even when allowance of 10% error is made for the colorimetric method of estimating the blood sugar content.

The Relation of Cholesterol with the Variations of Blood Fat Content.

Terroine (1914) found that the ratio of fatty acid to cholesterol was almost constant during the absorption/

absorption of fat.

This was not substantiated by Bloor (1915), who found the variations of cholesterol during fat absorption to be irregular, small, and sometimes barely appreciable. Other observers (Hiller, Linder, Lundsgaard and Van Slyke, 1924) have confirmed Bloor's observation.

The present conception of the behaviour of cholesterol during the absorption of fat, stated briefly, is that up to a certain point the cholesterol tends to increase, but not beyond this level, depending, perhaps, on the amount available in the intestine from food or bile. The association of cholesterol with fat does not appear to be essential either to the process of absorption or of transfer from blood to tissues.

In the present series of cases where the cholesterol content was estimated it is necessary to modify the figures on account of the colorimetric method of estimation. This means an error either way of 10%, and worked on this basis it will become evident that there is nothing conclusive in the variations - indeed, it may be deduced that the cholesterol content is unaffected, although it would be unwise to state definitely that no change occurs.

Results/

RESULTS

Section III.

Blood Fat and Exercise in Diabetes.

Section III. Blood Fat and Exercise in Diabetes.

Preliminary experiments directed towards the investigation of the constancy of the blood fat fasting content in patients suffering from diabetes mellitus gave the results shown in Table IX.

The results of the effect of exercise are shown in Table X. In certain cases the same amount of exercise has been performed (1) in the fasting state, (2) after the injection of 10 units of insulin. In all cases specimen A is a basal fasting sample. In the experiments where insulin has been given the 10 units are injected immediately after the collection of specimen A, and specimens B and C are collected at intervals of $\frac{1}{2}$ and 1 hour respectively, the patient resting in the interval. Specimen D is in all cases the sample immediately after exercise. Specimens E and F are collected at the times noted with the patient resting after exercise.

The clinical details of the subjects of the experiments are detailed immediately after the Table in which they are mentioned.

Figs. XI-XVIII represent in graphic form the changes detailed in Table X. The number of the figure is given in the right hand column of Table X opposite to the corresponding case.

Blood fat is in all cases represented in green and blood sugar in red.

Table IX.

Subject	Date	Fat Fasting Level. mg. per 100 cc.
J.B.	16.6.27	828.8
	9.5.27	731.1
F.T.	16.5.27	1130.7
	15.6.27	1425.6
D.T.	26.11.26	1050.8
	6. 6.27	982.7
	27. 6.27	1130.7
G.C.G.	16.12.26	902.8
	13. 4.27	577.2
	1. 6.27	982.7
	9. 6.27	879.1

Showing the lack of constancy of blood fat fasting content taken from the same cases on two or more occasions.

For clinical notes of cases in above Table IX see the clinical data of Table X.

Table X.

Case	Specimen.	Remarks	Time	Fat(mg. per 100 cc.)	Sugar(mg. per 100 cc.)	Work Done	Type of Experiment.	Graph
(1) G.C.G.	A	Fasting	10.5 a.m.	577.2	163.9	Bicycle Ergometer. Work done = 28,404 kg. metres	Without insulin	See Fig.XI
	D	After exercise	10.25	236.8	137.0			
	E	Resting	11.30	239.7	141.0			
13.4.27	F	Resting	12.30p.m.	91.7	133.3			
(2)	A	Fasting	10.20a.m.	879.1	129.0	Bicycle Ergometer. Work done = 28,404 kg. metres.	With insulin	See Fig.XII
	B	-	-	-	-			
	C	1 hr. after insulin	11.40	654.1	82.0			
	D	After exercise	12.10p.m.	932.4	83.7			
9.6.27	E	Resting	1.10	716.3	93.4			
(1) F.T.	A	Fasting	10.30a.m.	1130.7	400.0	Bicycle Ergometer. Work done = 5,257 kg.metres.	Without insulin	See Fig.XIII
	D	After exercise	10.50	1006.4	434.7			
	E	Resting	11.5 a.m.	873.2	454.5			
16.5.27	F	-	-	-	-			
(2)	A	Fasting	10.5	1425.6	351.0		With insulin	See Fig.XIV
	B	-	-	-	-			
	C	1 hr. after insulin	11.5	1317.2	283.8	do.		
	D	After exercise	11.15	1702.0	258.0			
	E	Resting	-	-	-			
(1) J.B.	A	Fasting	10.20	731.1	229.8	Bicycle Ergometer. Work done = 5,737 kg.metres	Without insulin	See Fig. XV
	D	After exercise	10.30	651.2	196.0			
9.5.27	E	Resting	11.20	301.9	224.8			
(2)	A	Fasting	10.15	828.8	175.4		With insulin	See Fig. XVI
	C	1 hr.after insulin	11.20	710.4	166.0			
	D	After exercise	11.30	1021,2	156.8	do.		
16.6.27	E	Resting	11.45	784.4	158.7			
(1) D.T.	A	Fasting	10.20	982.7	227.2	Bycycle Ergometer. Work done = 18,645 kg.metres	Without insulin	See Fig.XVII
	D	After exercise	10.45	908.72	230.0			
6.6.27	E	Resting	12 noon	799.2	205.2			
(2)	A	Fasting	11.20a.m.	1130.7	345.0		With insulin	See Fig.XVIII
	C	1 hr.after insulin	12.20p.m.	982.7	308.0			
	D	Afterexercise	12.35	1361.6	286.0	Do.		
27.6.27	E	Resting	-	-	-			

117.

Table X (contd.).

Case	Specimen	Remarks	Time	Fat(mg. per 100 cc.)	Sugar(mg. per 100 cc	Work Done	Type of Experiment.	Graph
(1) S.M. 6.5.27	A D E	Fasting After exercise Resting	10.10a.m. 10.40 12 noon	991.6 701.5 346.3	181.8 149.3 132.0	Bicycle Ergometer. Work done = 19,175 kg.metres	Without insulin	—
(1) W.C. 10.6.27	A D E	Fasting After exercise Resting	10.5 a.m. 10.20 10.50	976.8 962. 0 873.2	180.6 186.0 191.4	Bicycle Ergometer. Work done = 3,390 kg.metres	Without insulin	—
(1) H.V. 17.5.27	A D E	Fasting After exercise Resting	10.30 10.45 11.15	695.6 281.2 612.7	122.7 136.0 131.5	Bicycle Ergometer. Work done = 12,094 kg.metres	Without insulin	—
(1) W.T. 27.5.27	A D E	Fasting After exercise Resting	10.10 10.27 11.20	784.4 902.8 932.4	188.7 215.0 112.3	Bicycle Ergometer. Work done = 9,826 kg.metres	Without insulin	—
(1) G.C.G. 1.6.27	A D E	Fasting After exercise Resting	10.10 10.30 11.30	982.7 710.4 399.6	122.0 105.0 -	Bicycle Ergometer. Work done = 28,404 kg.metres	Without insulin	—
(1) D.T. 26.11.26	A D	Fasting After exercise	10.30 10.50	1050.8 606.8	253.0 255.0	Bicycle Ergometer. Work done = 40,965 kg.metres	Without insulin	—
(2)	C D	1 hr. after insulin After exercise	1.10p.m. 1.20p.m.	1038.9 1260.9	267.0 235.0	Bicycle Ergometer. Work done = 13,655 kg.metres	With insulin	—

Clinical Date - Table X.

Case G.C.G. 13.4.27. Out-patient of diabetic clinique of R.I.E. where he has been attending since March 1925. Has had insulin on and off since this date. An intelligent patient (medical student) and is now sufficiently conversent with the diet to guess weight of foodstuffs. Not on insulin at time of examination and guessing his diet of D 28.
Weight 11st. 9lbs.
His correct weight for height and age should be 11st. 4 lbs.

Case F.T. 16.5.27. Out-patient of diabetes clinique of R.I.E. where he has attended since 12th Oct. 1926. Diabetic symptoms started 1924 when he was treated by diet and insulin at the Leith Hospital.
At time of examination on diet C 19a and taking insulin in three doses of 19 units each day. (No insulin taken on mornings of examination).
Weight 11st. 4 lbs. Correct weight should be 10st. 10 lbs. Age 27.

Case J.B. 9.5.27. Out-patient at diabetic clinique R.I.E. since February 1922. Previously treated in ward 26 for gangrene of toe late in 1921.
At time of examination on diet C 28a and insulin in three doses of 14, 11 and 14 units. (No insulin taken on morning of examination).
Weight 12st. 5 lbs. Age 62.
Correct weight should be 11st. 13 lbs.
Later gangrene of the small toe(right) started but cleared up under treatment.

Case D.T. 26.11.26. An interesting case first met in Ward 32 in 1925 where he was admitted with underclothing stiff with sugar and was passing $\frac{3}{4}$ lb. of sugar a day until insulin was given. He cleared on 45 units of insulin. Attended as out-patient at diabetic clinique from October 1925. Has been/

Case D.T. (contd.)

been in and out of coma frequently and also various hypoglycaemic attacks.
At time of examination was on diet C 21a and two 9 unit doses of insulin.
Weight 11st. 4½lbs. Age 15.
Correct weight should be 8 st. 12lbs.

6.6.27. At the time of this examination patient was on diet C 22b and insulin in two doses of 15 and 16 units. His weight was 11st. 7 lbs.

Case S.M.

6.5.27. In-patient in Ward 26, R.I.E. in March 1927 complaining of thirst and a frequency of micturition of 6 weeks' duration. Showing sugar and acetone in the urine which cleared up on 45 units of insulin and a diet of B 15. After two months treatment he was discharged on a C 26 diet and 30 units of insulin.
At time of examination on ordinary diet with no bread and just finished a course of synthalein (1 week).
Weight 7st. 11½ lbs. Age 16.

Case W.C.

10.6.27. A history of 5 years standing treated previously at Swansea. Attended diabetic clinique first on 6th April 1927. At time of examination on diet C 21 and 10 units of insulin before breakfast (omitted on day of examination).
Weight 10 st. 3 lbs. Correct weight should be 12 st. 1 lb.
Age 49. Takes a good deal of C_2H_5OH.

Case H.V.

17.5.27. A new case reporting at the clinique for the first time. Complains of no thirst or polyuria but sugar discovered accidentally in the Materia Medica Department of the University. His father suffers from diabetes. On examination he had a trace of sugar since when he has never shown either sugar or acetone. He is now on an all round diet and shows no sign of diabetic symptoms. His weight was 9st. 8lbs. and his correct weight should be 9st. 8 lbs. Age 17 years. The initial glycosuria was probably nervous in origin.

Case W.T. 27.5.27. Another new case reporting at the clinique for the first time complaining of polyuria, no excessive thirst but some frequency of micturition. He showed a trace of sugar and a good deal of albumen in his urine. He had been on ordinary diet prior to the examination. The last remarks in his case suggest that the glycosuria was due to nervousness and that the cause of his symptoms might possible be nephritic in origin.

Table XI.

Showing the effect of the administration of 50 grams of glucose on the fasting blood fat content of the diabetic during rest.

Case	Date	Remarks	Blood Fat mg. per 100 cc.	Blood Sugar mg. per 100 cc.
W.R.	13.12.26	Fasting	1228.4	142.9
		½ hr. after glucose	932.4	277.7
		1 hr. do.	1139.6	327.9
D.M.	14.12.26	Fasting	1346.8	138.9
		½ hr. after glucose	1050.8	303.0
		1 hr. do.	1216.5	392.1
G.C.G.	16.12.26	Fasting	902.8	108.0
		½ hr. after glucose	858.4	277.7
		1 hr. do.	858.4	185.0

Clinical Data.

Case W.R. — 13.12.26. Longstanding case of diabetes mellitus attending diabetic clinique of R.I.E. Blood sugar curve still rising after 2 hours.

Case D.M. — 14.12.26. Definitely established case of diabetes mellitus attending diabetic clinique of R.I.E.

Case G.C.G. — See notes in relation to this same case under Table X.

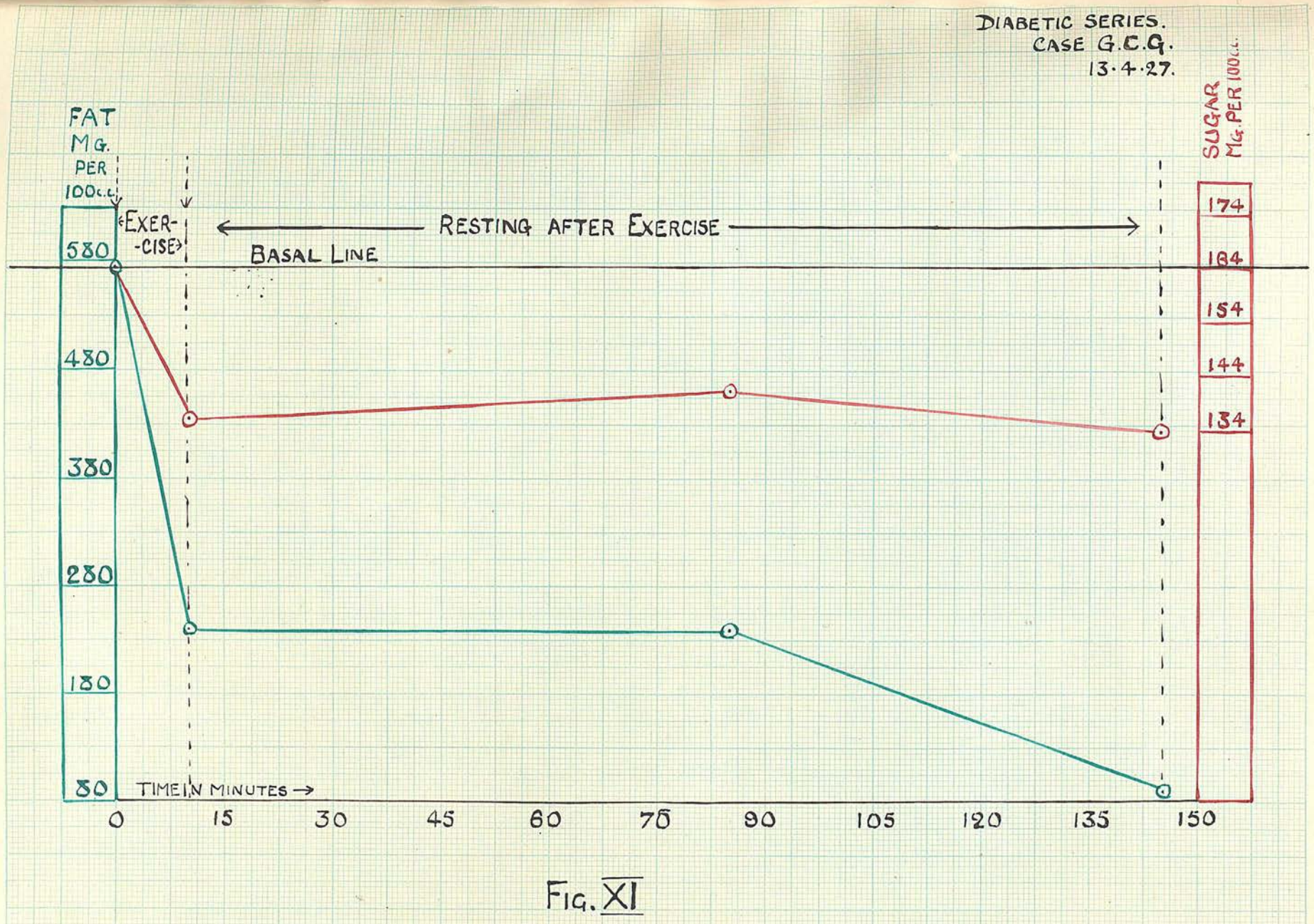

DIABETIC SERIES.
CASE G.C.G.
13·4·27.
FAT
MG.
PER
100c.c.
SUGAR
MG. PER 100c.c.
EXER-
-CISE
RESTING AFTER EXERCISE
BASAL LINE
580
480
380
280
180
80
174
164
154
144
134
TIME IN MINUTES →
0
15
30
45
60
75
90
105
120
135
150

FIG. XI

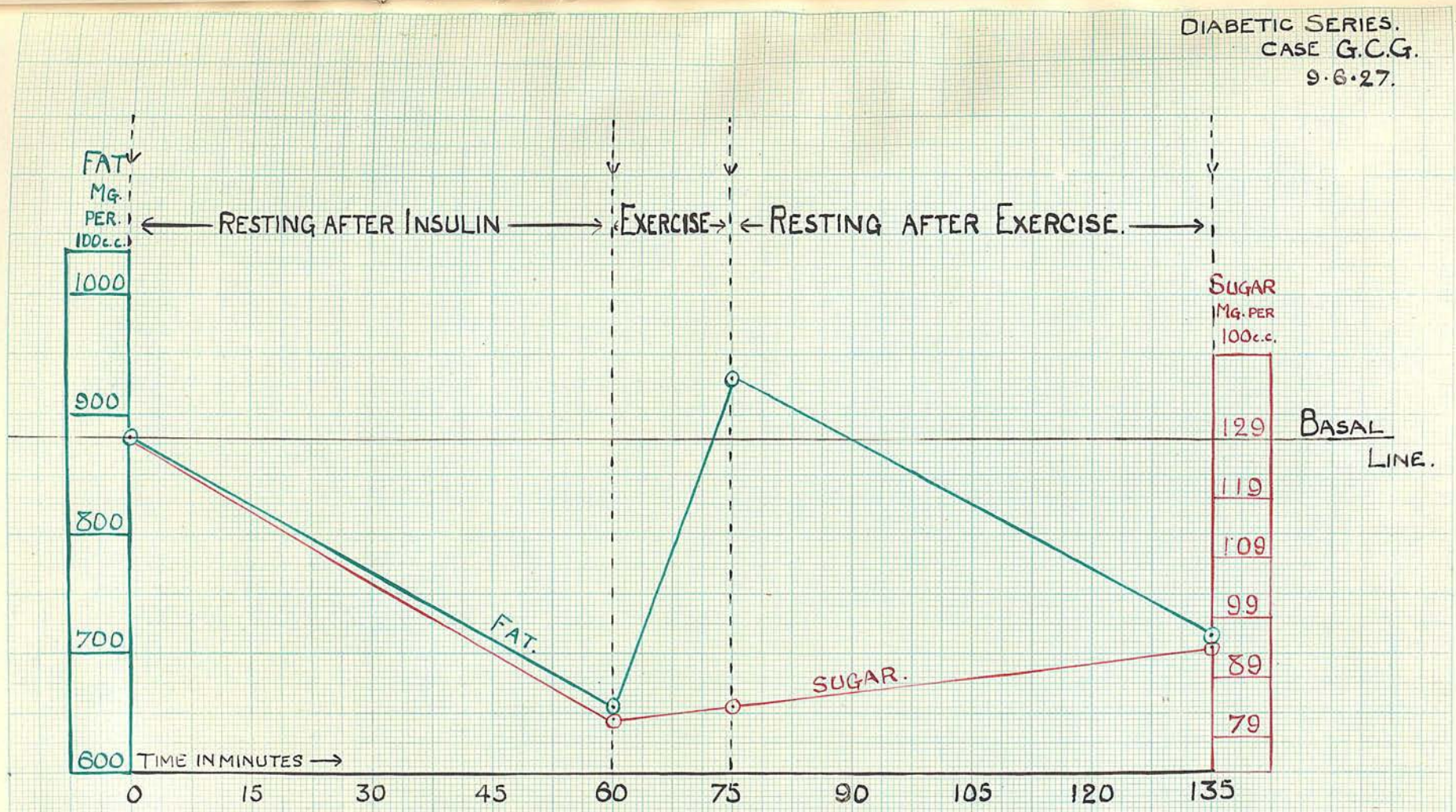

DIABETIC SERIES.
CASE G.C.G.
9·6·27.
FAT
MG.
PER.
100c.c.
1000
900
800
700
600
RESTING AFTER INSULIN
EXERCISE
RESTING AFTER EXERCISE.
SUGAR
MG. PER
100c.c.
129
119
109
99
89
79
BASAL
LINE.
FAT.
SUGAR.
TIME IN MINUTES
0
15
30
45
60
75
90
105
120
135

FIG. XII

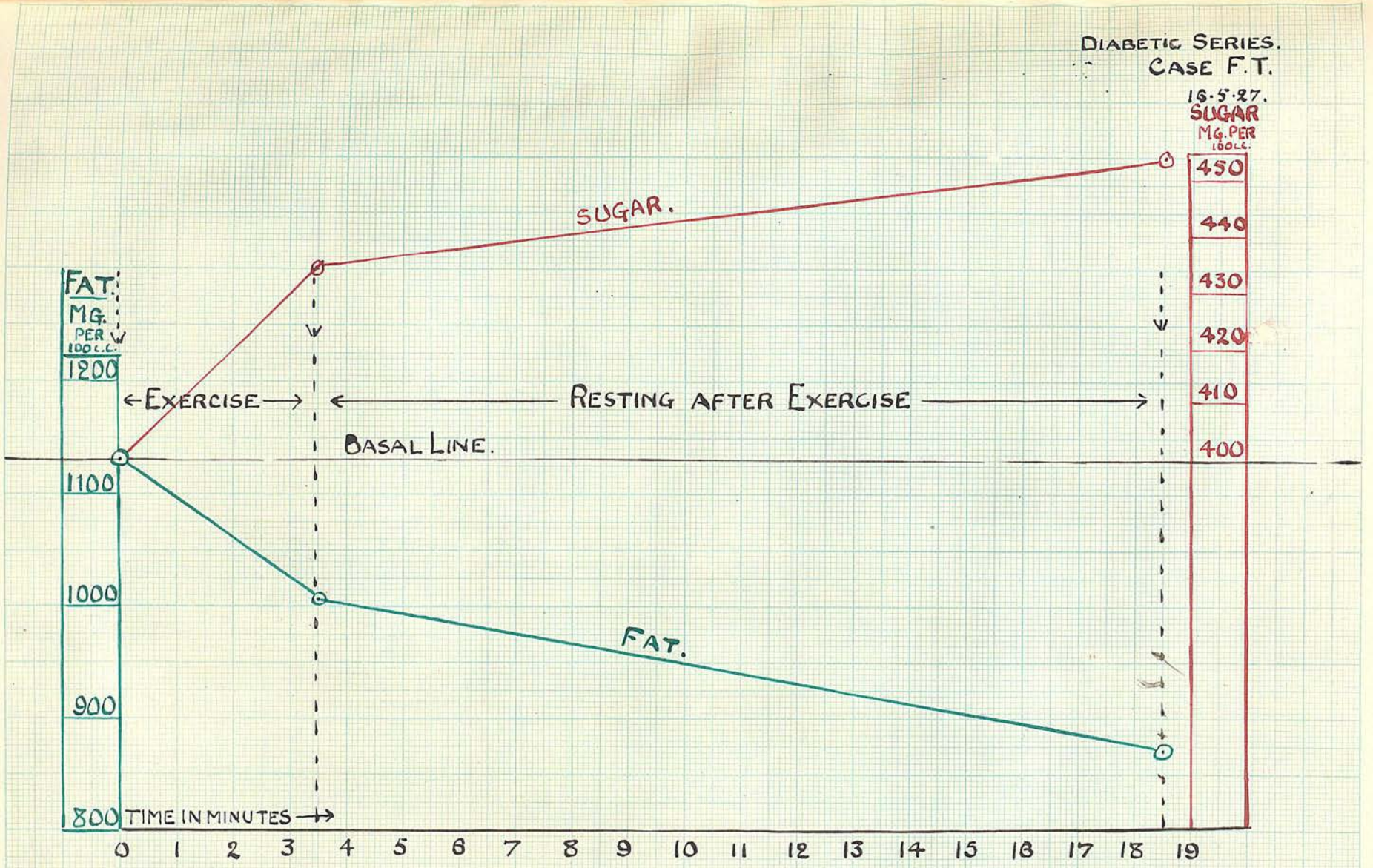

DIABETIC SERIES.
CASE F.T.
16·5·27.
SUGAR
MG. PER 100 C.C.
450
440
430
420
410
400
SUGAR.
FAT.
MG. PER 100 C.C.
1200
1100
1000
900
800
←EXERCISE→
←——— RESTING AFTER EXERCISE ———→
BASAL LINE.
FAT.
TIME IN MINUTES →
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

FIG. XIII

DIABETIC SERIES.
CASE F.T.
15·6·27.

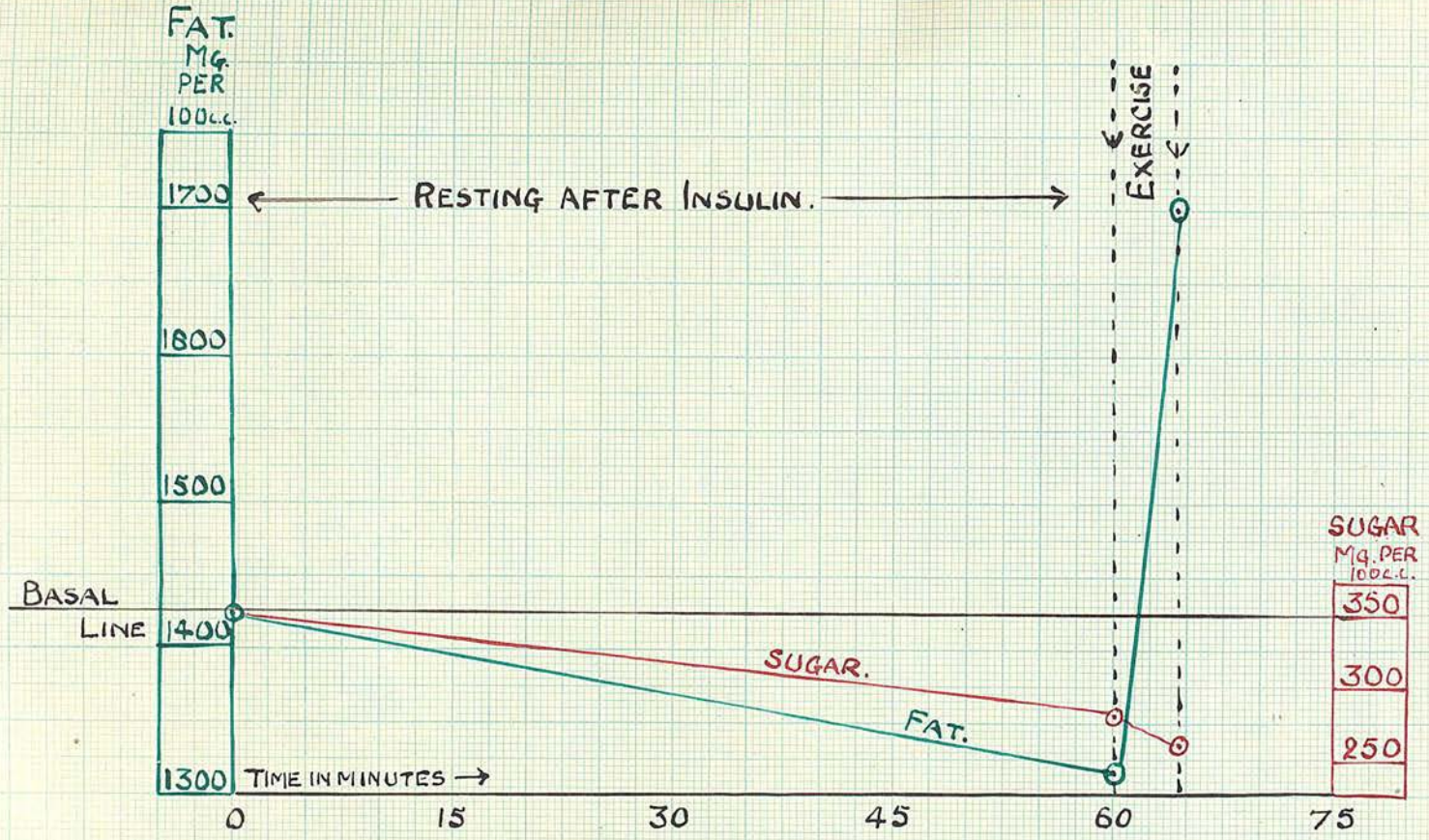


FIG. XIV

DIABETIC SERIES.
CASE J.B.
9.5.27.

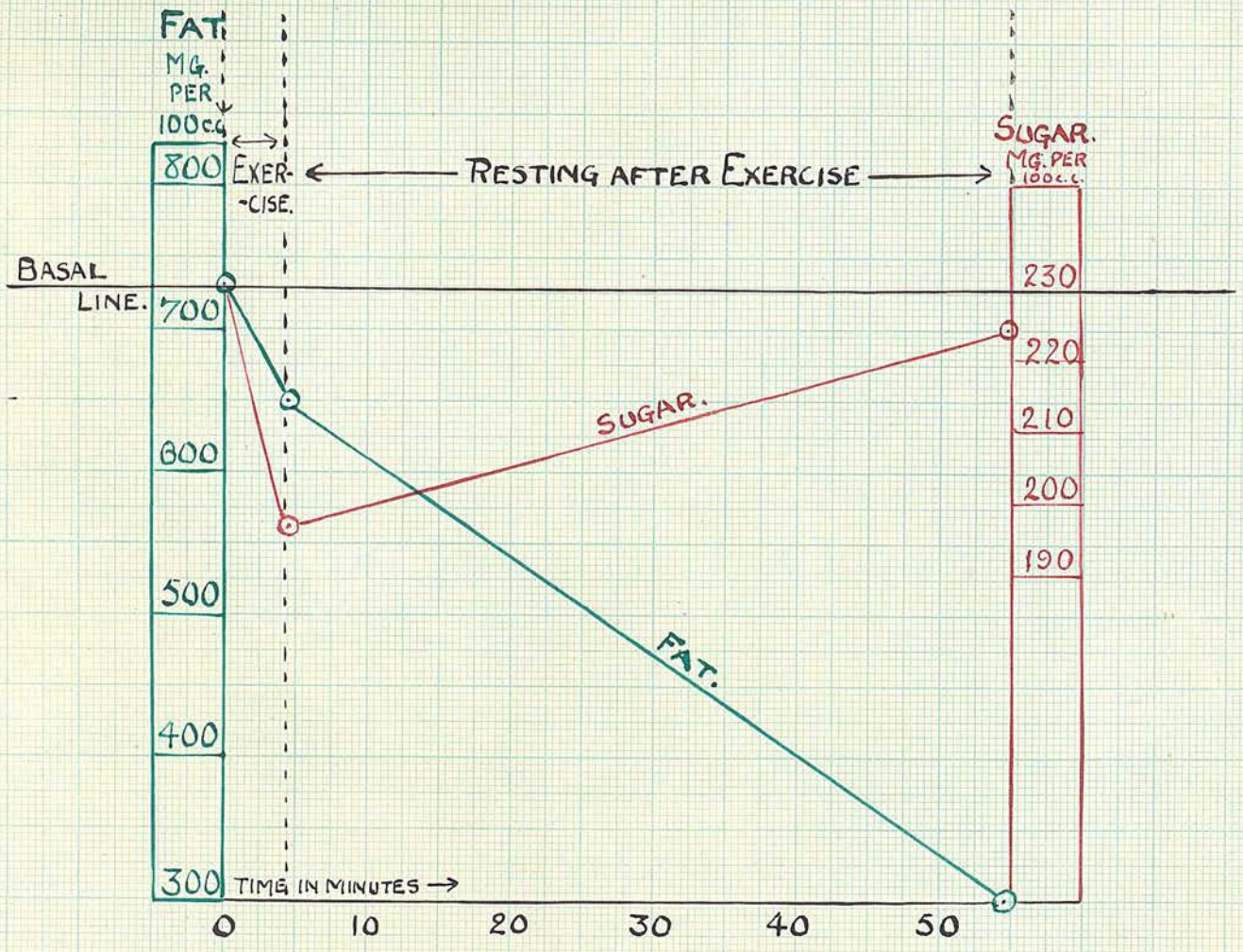


FIG. XV

DIABETIC SERIES.
CASE J.B.
16·8·27.

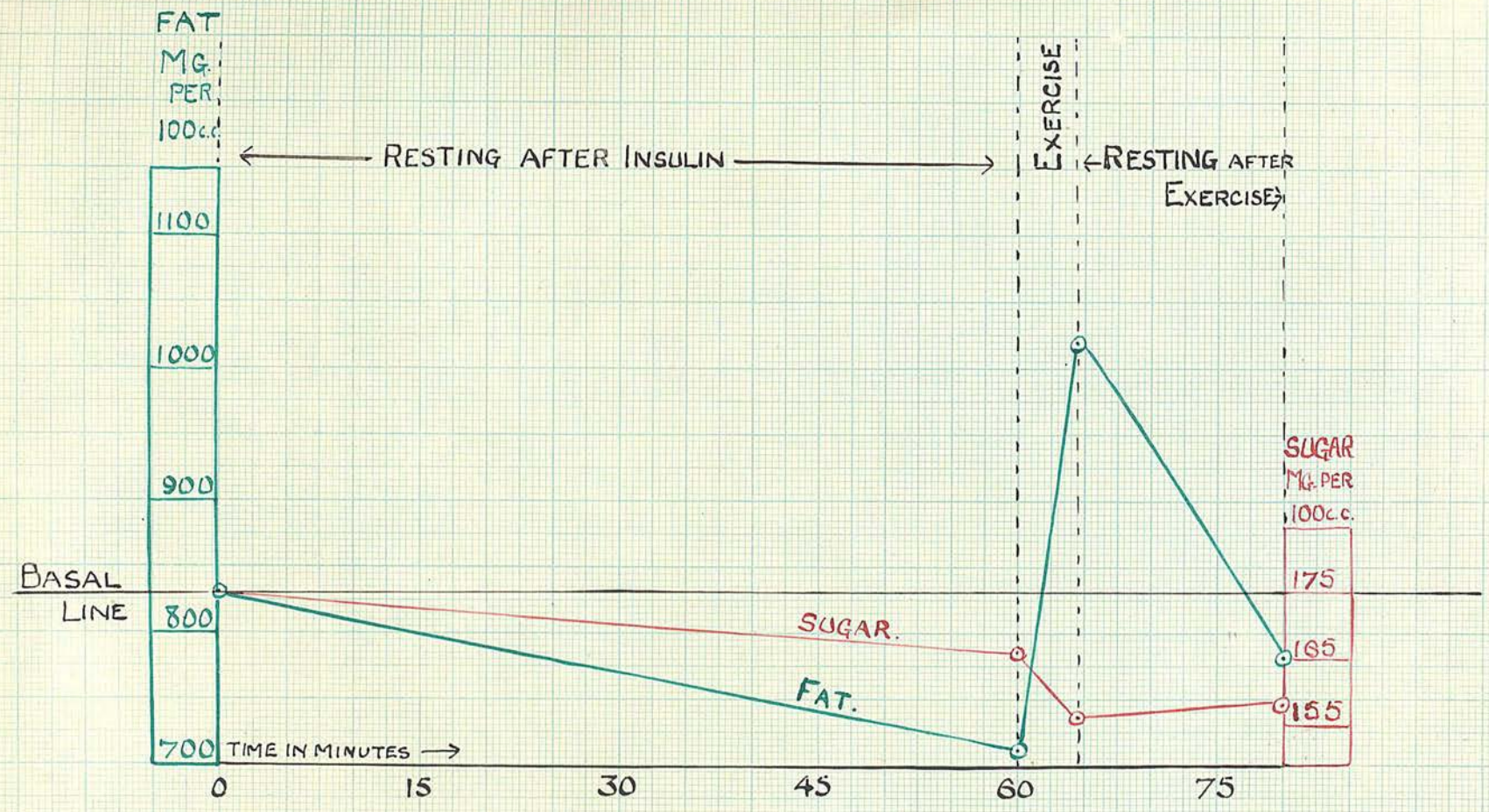


FIG. XVI

DIABETIC SERIES.
CASE D.T.
6·8·27.

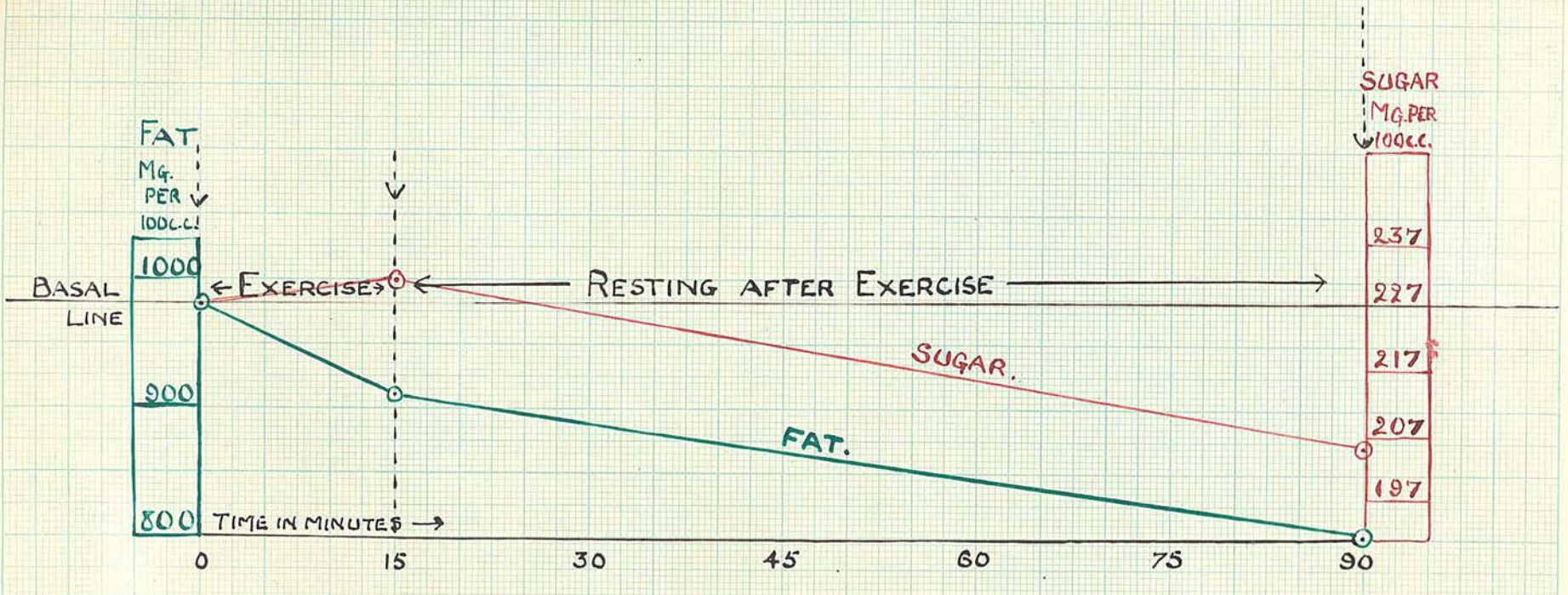


FIG. XVII

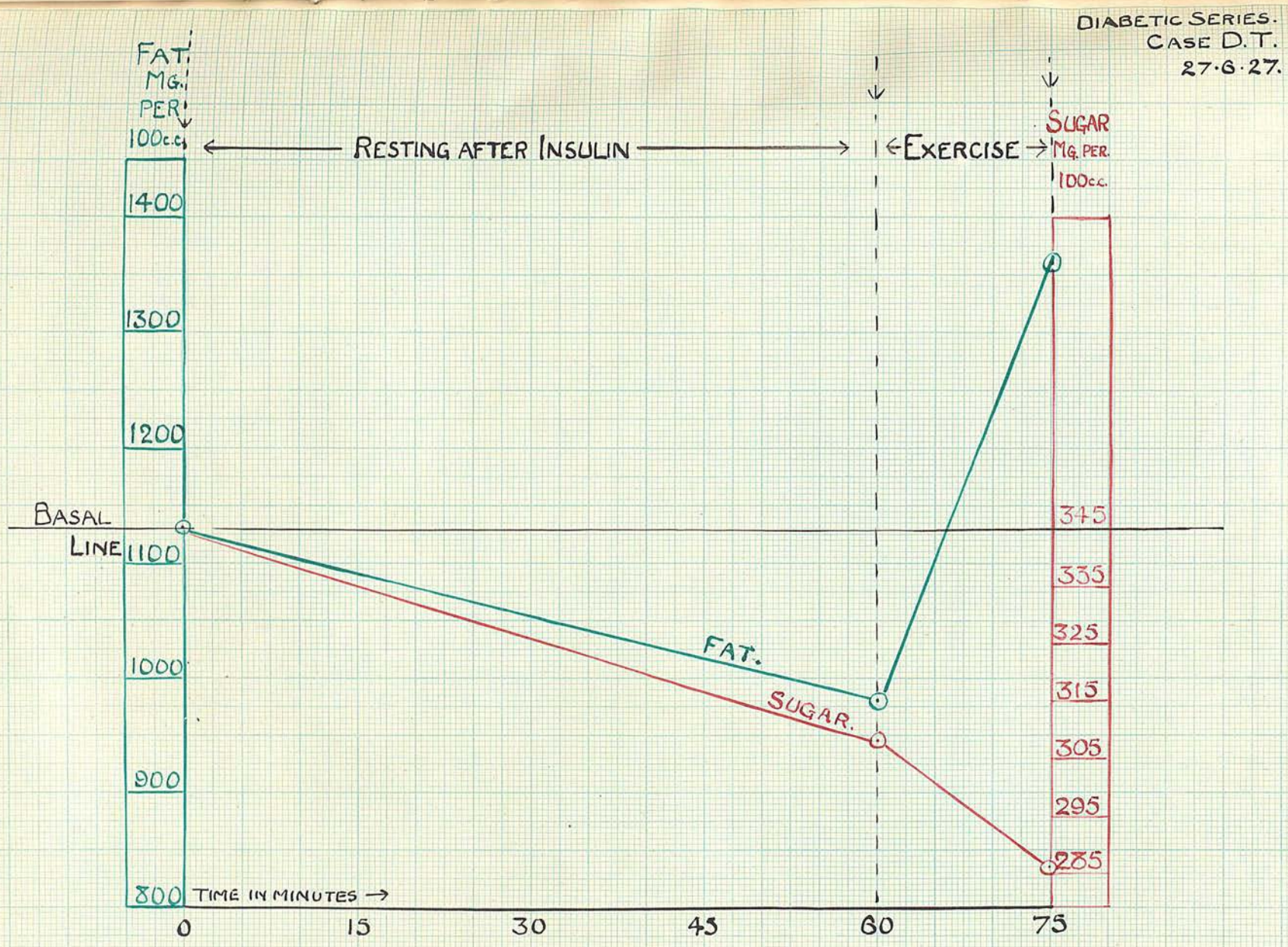

DIABETIC SERIES.
CASE D.T.
27·6·27.
FAT MG. PER 100c.c.
RESTING AFTER INSULIN
EXERCISE
SUGAR MG. PER 100cc.
1400
1300
1200
BASAL LINE
1100
1000
900
800
345
335
325
315
305
295
285
FAT.
SUGAR.
TIME IN MINUTES →
0
15
30
45
60
75

FIG XVIII

Discussion.

The outstanding feature of these series of experiments is the practically constant fall in the blood fat content after exercise in the fasting diabetic, which fall does not occur when the exercise is performed after an injection of insulin (Fig.12,14,16 and 18). Indeed after insulin the diabetic blood fat reaction approaches the response to exercise which we have seen in normals (Section II). This fall in the blood fat content is well beyond the margin of experimental error in most cases. Thus in case G.C.G. the blood fat content is decreased immediately after exercise by 58%, in F.T. by 10%, in J.B. by 10%, in D.T. by 7%, in S.M. by 29%, in W.C. by 1%, in G.C.G. by 27% and in D.T. by 42%. While some of these margins of percentage difference are not particularly marked (e.g. W.C. 1%), it must be emphasised that in all the cases quoted above the fall in blood fat is maintained during the period of observation so that in no case is the total fall in blood fat content less than 10%, while in one case (G.C.G.) the total fall amounts to 82%.

The question as to whether this variation is due entirely to variation of concentration of the blood consequent upon the exercise has been discussed in Section II, where it was shown that the increase in blood fat content after exercise in normals was far in excess of any concentration of the/

the blood which might occur. It was felt that investigation of one case along this line in connection with the diabetic series would suffice. G.C.G. shows a fall in concentration of corpuscles of 7% after exercise, while his blood fat content falls 58% so that one is now justified in claiming that the fall in blood fat after exercise is greater than can be accounted for by simple decrease in concentration of the blood and is a real and not an apparent decrease. (N.B.- Exercise effect on blood concentration usually results in an actual concentration of blood corpuscles which would make this decrease in blood fat content even more pronounced).

This variation in blood fat content after exercise in diabetics would be of greater significance if we could establish some constant blood fat content in diabetes mellitus which would serve as our"basal line", deviations from which could then be noted and due emphasis laid upon them. With this end in view fasting levels from different cases were withdrawn on two or more occasions. The results are shown in Table IX.

The Absence of Constancy of the Blood Fat Content in Fasting Diabetics.

It is obvious at once that the variation of the fasting content from day to day is more than can be accounted for by experimental error. This variation in the blood fat fasting level in diabetics

is/

is remarked upon by White (1926) who met with similar results under a similar technique. Since in pathological conditions, especially diabetes mellitus, there is no constancy of clinical manifestations but rather a daily variation, and since the metabolism of fat is so intimately concerned in this disease, it is scarcely surprising to find the results of chemical investigation so variable.

While the failure to show a constant fat content in diabetics similar to that found in normals (see Section II) may detract from the significance of blood fat variations after exercise, the fact remains that the fall of blood fat after exercise in diabetics is so constant and definite as to leave little doubt but that these factors are definitely associated as cause and effect.

The Fall in the Blood Fat Content in Fasting Diabetics after Exercise.

It will be noticed (Table X) that, with the exception of cases H.V. and W.T., the immediate effect of exercise in the fasting diabetic (where no previous injection of insulin has been given) is to reduce the level of the blood fat content and that this reduction continues for some time after the exercise has ended. Indeed, it is maintained in all cases throughout the period of investigation. The exceptions H.V. and W.T. were both new and untreated cases reporting at the diabetic clinique

of/

of the Royal Infirmary for the first time while all the other patients examined were long-standing and definitely established cases of diabetes mellitus.

Since the above experiments were carried out these two patients have been under observation and there is considerable doubt as to the accuracy of the original diagnosis. The one (W.T.) showed albumen in addition to sugar in his urine on first admission and when his albuminuria cleared up he became sugar free. The other patient (H.V.) whose blood sugar has never been really high came from a house where his father was suffering from diabetes mellitus and the nervous factor probably played a large part in the initial glycosuria.

Both cleared up rapidly on dietetic treatment and subsequent observation has, as has been noted, thrown doubt upon the diagnosis of diabetes mellitus. Of the two cases H.V. was the one who raised a suspicion that this might be a very early case and it is interesting to note that his fat reaction after exercise showed a definite immediate fall of 60% with a rapid recovery of the blood fat content within ½ hour after exercise. As we shall see in the advanced diabetic the fall in blood fat is maintained for some time after the exercise, but this immediate fall with rapid recovery may be the reaction of the early type.

With these two exceptions, then, the blood fat/

fat falls immediately after exercise and continues to fall during the period of observation of these experiments.

It would appear, therefore, that, in the diabetic, exercise calls for the withdrawal of fat from the blood and that processes are set in motion whereby that withdrawal continues for some time after the exercise has ceased. Apparently in these cases, the mechanism which normally exists for preserving a more or less constant blood fat content is upset. Thus normally we have seen that exercise effort is met by an apparent outpouring of fat with a resulting temporary increase in blood fat content. This is one of Nature's typical responses to a demand for any commodity and the demand of the working cells (in severe exercise) for fat is met by a generous response from the depôts whereby the drain on the fat in the blood consequent upon the exercise does not result in a lowering, and possible shortage, but rather in an increase with which to meet all emergencies.

This lowering of the blood fat which occurs in fasting diabetics after exercise may be interpreted in various ways. Thus the exercise done in many cases is so slight that it might be argued that the blood fat content of diabetics was sufficient to meet the demands of the working cells without calling out any help from the depôts and

hence/

hence the blood fat content falls without having required to send out demands to the depôts (a demand which is met in the normal by a great out-pouring of fat with a consequent rise in concentration in the blood). It should be noted that in the normal the average fasting fat content is 602 mg. per 100 cc. (Table IV, section 1), while that for diabetics is 973 mg. per 100 cc. (This latter figure is obtained from the average of the fasting levels shown in Table X with the exception of the two doubtful cases H.V. and W.T.). In the experiments on normals a quarter of an hour's strenuous exercise was performed by each subject, but with the diabetic series it was found that the amount of exercise possible varied in individual cases, some becoming exhausted rapidly after two or three minutes' effort. Consequently patients were instructed to perform as much exercise as they possibly could on the bicycle ergometer and the time taken was noted and the amount of work done calculated. In those cases where repeat experiments were made with a previous injection of insulin, the previous amount of exercise performed was repeated. In only one case (the patient G.C.G. who completed a full 15 minutes' effort) were hypoglycaemic symptoms encountered and these were of the mildest type.

Despite the fact, however, that the exercise performed by many of these patients was slight, two cases/

cases, viz. D.T. and G.C.G., although both definite diabetics, managed to complete the full 15 minutes' arduous exercise equally strenuous as that undertaken by any of the normals. In neither case was there any rise in the blood fat content in response to the exercise but rather a constant fall maintained throughout the period of observation. There is no question in these cases at least, but that the exercise was far in excess of that which could be maintained by the fat present in the blood without any additional supply from other sources. Consequently we must look for some other explanation of the fall in the blood fat level.

Could it possibly be that the explanation lay entirely in increased avidity of the working cells for fat and that the increased demand is in excess of the supply in these cases although the response to the demand is still of normal degree?

There is no doubt that such a possibility exists but it must be pointed out that while the efficiency of fat for exercise may not be so complete as that of carbohydrate, still it is fairly efficient and one would expect that, provided the supply were fairly constant, muscular exertion would be able to be maintained fairly well.

The striking factor in all these cases (with the exception of the cases D.T. and G.C.G. already mentioned) was the enormous difficulty these diabetic/

diabetic patients had of maintaining exercise effort for any length of time. Clinically, too, one of the commonest complaints of the diabetic is the muscular weakness and fatigue following slight exertion.

It would appear then that such a condition of muscular fatigue would be more liable to occur with a definite deficiency of supply of fat rather than by a normal supply response and increased avidity of the working cells. No doubt the two factors are associated in the diabetic, but it would appear that there is a definite lack of response whereby the demand of the working cells, either normal or increased, is not met.

Effect of Insulin Injection on the Fasting Blood Fat Content of Resting Diabetics.

In those cases where the reaction of the blood fat to exercise after the injection of 10 units of insulin was being investigated, it was considered necessary to keep the patient resting for one hour after his injection before performing his exercise so that the action of the insulin might have a chance of asserting itself.

The procedure adopted was to withdraw a fasting sample after adequate rest as already indicated, followed by the injection of insulin (10 units) and a further period of rest of one hour's duration. At the end of this period the second

sample/

sample was withdrawn and immediately afterwards the exercise was imposed. From these two samples it was possible to determine what effect, if any, the injection of insulin had on the blood fat of diabetes during absolute rest.

Reference to Table X will show that in the first four cases, (1) G.C.G. with insulin, (2) F.T. with insulin, (3) J.B. with insulin, and (4) D.T. with insulin, the blood fat in every case fell during this resting phase (i.e. specimens A and D).

Thus G.C.G. falls 25%, F.T. 7%, J.B. 14% and D.T. 13%; all definite falls from the previous fasting levels and well outside the margin of experimental error.

This reduction has been noted by other observers and Macleod and Campbell (1924) state that insulin reduces the fat content of both liver and blood in experimentally produced diabetes.

There seems to be little doubt that insulin does act in this way when the blood fats are fairly high as occurs in diabetes mellitus. Davies et alii (1923) noted that by the naked eye a marked reduction in the lipaemia of a severe case of acidosis was evident three hours after the injection of insulin.

This effect of insulin on the blood fat content is particularly interesting in view of the prompt effect of the injection of insulin on the excretion/

excretion of ketone bodies.

Whether insulin acts directly upon the fats or not is a question which cannot be decided lightly. There is no doubt that the metabolism of fat and carbohydrate are intimately associated and it is a well known fact that ketone bodies appear as a result of the incomplete metabolism of fat whenever an insufficiency of carbohydrate occurs in the simultaneous combustion in the animal organism.

It has further been indicated in Section II that the administration of glucose produces an immediate reduction in the blood fat in normals during the first half hour of absorption and Table XI shows a similar state of affairs to exist in association with diabetes. In these three cases 50 grams of glucose were given after the withdrawal of the fasting blood sample and thereafter two samples were collected at intervals of half an hour, the patient resting during the observations.

It will be noted that at the end of the first half hour the blood fat content has fallen but rapid recovery occurs in the first two cases, at least.

Oliver and Haworth (1923) noted a somewhat similar inter-relationship between the administration of glucose and the variations in blood fat as above, but in their experiments the recovery of the blood fat level was not so early, being delayed for about an hour and a half.

These observers made the interesting observation that the reverse of the above experiment is also true, viz. that administration of fat (in the form of butter) to a diabetic results in a lowering of the blood sugar curve. They suggest that the fall in blood sugar after fat suggests (a) either increased glucose needed for the oxidation of the fat or (b) some inhibitory mechanism has been brought into play leading to increasedglucose storage.

It is quite possible that the effect of insulin on the ketone bodies may be indirectly through its action on the carbohydrates whereby the carbohydrate metabolism is improved. Such a view is supported by the experiments of Hirschfelder and Maxwell (1924) where insulin was shown to have no effect in reducing the toxicity of acetone bodies when these are administered to experimental animals.

Similar results had been previously reported by Robertson and Anderson (1923).

On the other hand Raper and Smith (1925) found a migration of fat from liver to muscles occurred when extreme effects of insulin on the blood sugar were produced. Such changes occurred only when hypoglycaemia was produced and was evidenced in an increase of blood fats.

In considering results of animal experimentation in this connection, it would be well to point out that the conditions in normal animals are fundamentally/

fundamentally different from those occurring in a depancreatised animal for whereas in the latter the injection of insulin only restores to the body a substance in which it is deficient, it adds an extra supply to the already sufficient amount present in a healthy animal and so upsets the normal metabolic balance, thereby inducing an abnormal state.

The metabolic derangement consequent upon wholesale evisceration and decerebration (so popular among some observers) must be tremendous and the results of such investigations seem hardly likely to yield data of any permanent value.

The Response of the Blood Fat of the Diabetic to Exercise after Insulin.

Referring again to Table X it will be seen that when the same exercise is repeated one hour after the previous injection of insulin the response of the blood fat is entirely changed.

It now becomes the type of response found in normals in so far as instead of a fall of blood fat occurring after exercise a definite rise takes place.

Apparently, then, from our argument of the previous paragraph insulin has restored the impaired functioning capacity of those organs whose duty it is to respond to the demand of the working cells for fat by an outpouring of this foodstuff into/

into the blood stream.

Such "organs" are the fat depôts and the liver and it would appear possible that, in the absence of insulin, the desaturation processes of the liver are arrested in varying degree, depending on the severity of the case, and that the fat is thus dammed up in this organ.

It is possible that the condition of the liver with regard to fat content might regulate the rate of discharge of fat from the depôts and that the arrest of the fat processes in the liver would mean the cessation of impulses to the fat depôts for discharging fat. Such a theory would explain the pre-diabetic fat type of patient where insufficiency of insulin would result in a partial arrest of desaturation processes in the liver and resulting lack of stimulus to the fat depôts to discharge their stores with consequent accumulation in such depôts amounting to obesity.

Such a conception would also account for the occurrence of large accumulations of fat in the livers of diabetics found post-mortem and would fall into line with the findings of Macleod and Campbell (1924) and Raper and Smith (1925).

The increase of blood fat which is so constant a feature of diabetes mellitus (we have already noted the average fasting fat levels of diabetics/

diabetics to be 973 mg. per 100 cc. as compared with 601 mg. per 100 cc. found in normals) might be the result of this damming back action of the liver whereby neutral fat from the depôts (with long C chains) supplies the greater part of the circulating blood fat. This would appear quite logical since fat is normally poured out from the depôts and taken, presumably to the liver, for desaturation processes. It may be that the control over the discharge of fat from the depôts is maintained by the liver's rate of desaturation and the consequent level of fat content in the blood. When the fat content of the blood falls the fat depôts will release their contents and when it rises their rate of discharge will be retarded.

In an advanced case of diabetes with practically complete damming up of the liver owing to lack of insulin it is possible that the body takes on a new rôle as far as the metabolism of fat is concerned and determines to metabolise fat (for its cells must live and carbohydrate cannot be utilised by them) without the assistance of the liver. Thus the long-chained neutral fats from the depôts are called into service and are metabolised possibly by direct β-oxidation and necessarily must pass through the 4 C chain stage, at which danger point ketone bodies are liable to occur.

The desaturation processes occurring normally/

normally in the liver may well be devised so as to avoid any such danger point on the line of the oxidation of fat.

The immediate effect of the injection of insulin will be to re-open the liver route of fat metabolism whereby desaturated compounds are released once again to the tissues and these desaturated compounds, forming a more "palatable" commodity for the tissues are taken up by them, in preference to the compounds of the more direct oxidation described above, resulting in a rapid disappearance of the ketone bodies.

Hirschfelder and Maxwell (1924) and other observers (Robertson and Anderson, 1923) have found that insulin has no direct action on reducing the toxicity of acetone bodies administered to experimental animals and, while the action of insulin in reducing in the diabetic may be due to the indirect effect of improved carbohydrate metabolism as these observers would believe, it appears that, under the theory of insulin action here presented, no effect on the toxicity of acetone bodies would be expected since in their experiments the ketosis is not due to liver dysfunction consequent upon lack of insulin.

Clinically it is quite common to meet with the type of diabetic where the sugar loss is more or less easily controlled by diet but where acetone persists/

persists no matter how you balance your diet or juggle with your G:F.A. ratio.

In such cases one might argue that surely the balancing of the diet and the clearing of the urinary sugar loss ensures that carbohydrate and fat metabolism are complete and that the ketosis is not due to interrupted fat metabolism consequent upon deficiency of available carbohydrate. Injection of insulin will always clear these patients of acetone and this would seem to point again to the liver derangement being removed by the insulin so that once again desaturated fats are set free for tissue use.

In other words this type of case would lead one to suppose that there is some additional factor in the production of ketosis over and above simple carbohydrate deficiency and that the additional factor may be found in the incapacity of the liver to deal with fat reaching it from the depôts due to deficiency of insulin and the consequent, what one might term "direct β-oxidation" of fat from the depôts, as described above.

The metabolism of all three foodstuffs is so closely connected and their inter-relationship one with another, so intimate that the concentration and balance of the one cannot altered in any degree without the reflex of such alteration manifesting itself/

itself in the other two. Hence it would appear logical to assume that any controlling factor such as the internal secretion of the pancreas which exerts a definite action on one constituent should also exert some controlling influence on other constituents. Thus while the main object of control under insulin may be that of the carbohydrate metabolism, it does not necessarily follow that its controlling influence is restricted to carbohydrate. Nor, indeed, does the fact that the carbohydrate control by insulin is its most obvious action exclude the possibility of its being of secondary, rather than primary, importance. To assume that insulin has some direct action on fat metabolism does not, per se, deny its action on carbohydrate but merely suggests an additional action.

In the present state of knowledge this explanation of the experimental results recorded in this paper is merely a hypothesis affording a working basis for further investigation. In particular valuable supporting evidence (or, of course, the reverse) would be afforded by a research concerning the relative degree of unsaturation of liver fat in normal and diabetic subjects - in the latter case with and without the use of insulin.

It will be noticed that the hypothesis under consideration/

consideration demands that in diabetic subjects the liver fat shall be less unsaturated (i.e. shall have a lower iodine value) than in normals but that administration of insulin shall cause the restoration or normal degree of unsaturation. So far as the author can ascertain this point seems to have escaped investigation almost completely.

Allan, Bowie, Macleod and Robinson (1924) have found that in depancreatised dogs the liver fat is quantitatively increased (though they do not appear to have noted its iodine value) and that administration of insulin causes first a disappearance of this extra fat with later fatty degeneration of the liver.

Little, of course, can be founded on this since depancreatisation does far more than merely remove the insulin supply, but on the whole it does appear slightly to support the present hypothesis.

On the other hand Marrian and Dudley (1923) find that administration of insulin to normal animals (mice) is followed by no change in the liver fat either as regards quantity or iodine value.

Hepner and Wagner (1927), however, disagree with these investigators for in similar experiments with dogs, rats and guinea pigs, they find that the fatty acids of the liver became less saturated.

Had/

Had this last result been obtained by the administration of insulin to deabetic animals, it would have been sufficient to disprove the hypothesis here advanced. Since, however, an excess of insulin may be conceived as acting in more than one way even on the basis of the carbohydrate; since, moreover, the results of Hepner and Wagner were obtained in hypoglycaemic animals and, finally, since their results do not agree with those of the similar experiments of Morrian and Dudley, there seems to be no adequate reason for abandoning the hypothesis as yet.

General/

General Summary of Results.

Section I.

(1) Figures are presented (Table IV) showing the average fasting fat content of the blood in normal individuals to be about 600 mgms. per 100 c.c. (with a range between 439 and 698).

(2) In obesity the fasting fat content is shown to be considerably higher, the average being 1744 mgms. per 100 c.c. (with a range of 1263 to 2619). See Table IV).

(3) In nervous diseases where muscular tremor is a feature the fasting fat content is remarkably low, the average being 306 mgms. per 100 c.c. (with a range of 180 to 449). See Table IV.

(4) Finally in Table V, figures are brought forward indicating the high fasting fat content found in patients who showed evidence of kidney impairment.

Section II. /

Summary. - Section II.

1. Figures are presented indicating a remarkable constancy of the fasting blood fat content for the same individual.

2. A definite increase in the blood fat content is noted as occurring in fasting individuals immediately after severe exercise.

3. There is no increase in blood fat after exercise in the same individuals when they have received 100 grams glucose per os prior to the muscular exertion.

4. The variations of the R.Q. are discussed and the suggestion made that, taken in conjunction with the observed increase in blood fat fasting individuals after exercise, they might indicate that fat was being mobilised for its conversion into carbohydrate.

5. Indications of a relationship between the blood fat and blood sugar contents are noted.

6. The investigation of the cholesterol content of the blood seems to indicate that during the period of observation it remained unaffected by the exercise and, apparently, unrelated to the definite blood fat variations.

Section III - Summary.

1. Figures are presented showing the absence of any constancy of the fasting blood fat content of diabetic individuals from day to day.

2. A definite decrease in the blood fat content is noted as occurring in fasting diabetes immediately after exercise.

3. There is no decrease in the blood fat after exercise in the same individuals, but a definite increase (approaching the response of "normals"), when they have received an injection of 10 units of insulin prior to the muscular exertion.

4. Indications of an apparent relationship between the blood fat and blood sugar contents in diabetics are noted.

5. The effect of the injection of insulin upon the fasting blood fat content of the diabetic in the resting state is described during the first hour of insulin action.

6. An hypothesis of the action of insulin in relation to blood fat is presented and discussed.

The/

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